CHANGES IN THE AMINO ACID COMPOSITION OF CHICKEN MEAT AND DRIP DURING FROZEN STORAGE

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY

Edward Joseph Wladyka

1965

This is to certify that the

thesis entitled

CHANGES IN THE AMINO ACID COMPOSITION OF

CHICKEN MEAT AND DRIP DURING FROZEN STORAGE

presented by

Edward Joseph Wladyka

has been accepted towards fulfillment of the requirements for

Ph.D. **degree in** Food Science

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Major professor

Date Nov. 10, 1965

O-169

CHANGES IN THE AMINO ACID COMPOSITION OF CHICKEN MEAT AND DRIP DURING FROZEN STORAGE

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Food Science

1965

ABSTRACT

CHANGES IN THE AMINO ACID COMPOSITION OF CHICKEN MEAT AND DRIP DURING FROZEN STORAGE

by Edward Joseph Wladyka

This study was undertaken to evaluate the effects of specific periods of frozen storage on the composition of essential amino acids in dark and white chicken meat and drip and the release of drip from dark and white chicken meat during thawing. The meat and drip from heavy hens were used in this study.

Larger quantities of drip were obtained from white meat than dark meat. The percentage drip obtained from white meat at 30 and 90 days was 5.4 and 9.6 percent respectively, while that obtained from dark meat was 4.8 and 7.9 percent, respectively. This also indicated that more drip was obtained from both dark and white meat after 90 days of storage than after 30 days.

Optimum conditions of hydrolysis for amino acids in chicken meat were determined by studying the effects of time and method of hydrolysis on the liberation of arginine, leucine and methionine. The methods of hydrolysis compared were refluxing with 6N hydrochloric acid, autoclaving in a flask at 15 pounds pressure with 2N hydrochloric acid, and autoclaving in a sealed tube with 3N hydrochloric acid. The quantities of amino acids were determined by microbiological assay using L. mesenteroides as the test organism. Maximum liberation of the three amino acids was obtained in 16 hours.

Drip from chicken meat was hydrolyzed by refluxing with 6N hydrochloric acid; the optimum release of arginine, leucine and methionine was obtained between 8 and 16 hours of hydrolysis. A 16 hour hydrolysis period was used for both chicken meat and chicken drip.

More protein (protein nitrogen) was found in the drip from dark meat than in the drip from white meat. The protein content of drip obtained from white meat after 30 and 90 days of frozen storage was 9.17 percent and 10.92 percent respectively; the content in drip from dark meat after 30 and 90 days of frozen storage was 5.16 percent and 6.26 percent, respectively. Drip obtained from dark and white chicken meat after 90 days of frozen storage also contained more protein than the drip from meat held in frozen storage for 30 days.

The essential amino acids in acid and alkaline hydrolyzates of dark and white chicken meat and drip were determined by microbiological assay. L. mesenteroides was used for determining arginine, leucine, isoleucine, valine, lysine, histidine, methionine, and phenylalanine; L. arabinosus was used to determine tryptophane; and threonine was determined using S. faecalis. Acid hydrolyzates from drip were also analyzed with an amino acid analyzer.

Percentage of the essential amino acids in both dark and white chicken meat decreased as the period of frozen storage was extended. The concentrations of essential amino acids in the drip from dark and white chicken meat increased as storage time increased. Larger quantities of the essential amino acids were detected in the drip from white meat than from dark meat after both periods of storage. Similar results were obtained by both the microbiological assay and the amino

acid analyzer.

The quantitative results obtained from the analyzer were higher than those obtained by the microbiological assay and the differences were attributed to the loss of protein nitrogen on decolorizing the hydrolyzates prior to microbiological assay.

The proportions of amino acids in drip were similar to the proportions of the amino acids in the frozen meat. This may have indicated a denaturation of the structural protein in the frozen stored meat.

The results obtained under the conditions of this study indicated that the practice of holding poultry for extended periods of frozen storage resulted in protein breakdown and the subsequent loss of proteins by leaching.

ACKNOWLEDGMENTS

The author is sincerely grateful to Dr. L. E. Dawson for his guidance and council throughout the author's course of study and constructive criticism during the development of this research project; to Dr. B. S. Schweigert for his advice and cooperation in making facilities available for this study; to Dr. C. L. Bedford, Dr. A. M. Pearson, Dr. T. F. Irmiter and Dr. J. F. Price for their critical review of the manuscript.

The author wishes to thank Miss Doris Bauer for her advice and invaluable assistance in amino acid analysis and to Mrs. Maurice Ritchey for her help in preparing the manuscript.

To his wife Barbara, the author gives special thanks for her encouragement and understanding throughout a trying period.

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INTRODUCTION

In recent years, the practice of chilling poultry in ice-water mixtures has gained acceptance as a means of removing body heat to delay bacterial spoilage. Considerable quantities of water are absorbed by the poultry carcasses during chilling, thus, federal regulations have been established which specify limits for water absorption.

A large percentage of broilers are sold as ice-packed birds, although a considerable tonnage is frozen prior to distribution. Fresh fryers are frequently frozen in chest-type freezers after purchase by the consumer. Roasters and turkeys are usually packaged and frozen by the processor prior to sale. All frozen poultry may be held in storage for varying periods of time prior to consumption.

Considerable quantities of moisture are released from the carcasses upon thawing. Therefore, the ultimate consumer is confronted with the unsightly appearance of the product and also the unpleasant experience of removing the dripping carcass from the package. The possible loss of nutrients in the drip must also be considered. These problems are of considerable importance to the poultry industry.

A number of investigators have reported on the effects of various factors (rate of freezing, freezing temperature, thawing temperature, Pearson et al., 1959; Callow, 1952; Hiner, 1945) on the quantities of drip lost from animal tissues. Some workers have examined the formation of drip from frozen tissue in terms of the physico-chemical changes which occur in the frozen state. In recent years investigations have been concerned with the composition of drip and possible origin of the components of drip fluid. However, little

information is available on the nutritive value of drip.

While some investigations have been made on the amino acid composition of chicken, little information is available on the comparative concentrations of the essential amino acids in both dark and white chicken meat. In addition, no indication was found in the literature as to the effects of periods of frozen storage on the amino acid composition of dark and white chicken meat.

In the past, the use of microbiological assay methods provided a precise means of determining the amino acid composition of animal tissue. In recent years the amino acid analyzer has also proved effective in the quantitative determination of amino acids in biological materials. The usefulness and dependability of both methods of analysis have been confirmed by the work of a number of investigators.

The purposes of this study, therefore, were: 1) to evaluate the effects of specific periods of frozen storage on the amino acid composition of dark and white chicken meat by microbiological assay, 2) to determine the amino acid composition of drip obtained from frozen dark and white meat held for specific periods of time, and 3) to evaluate the effect of specific periods of frozen storage on the release of drip from frozen dark and white chicken meat.

LITERATURE REVIEW

Cook et al. (1926) defined drip as the clear, reddish-brown colored fluid which exudes from all cut surfaces of meat that have been frozen and thawed. They reported that drip contained approximately 9 percent protein and that the percentage protein was approximately the same for drip from either beef, lamb or pork.

Love (1955 a) referred to fish drip as the cloudy fluid exuding from fillets which were allowed to stand for some time under humid conditions. Sair and Cook (1938 a) stated that drip was the fluid which exuded after freezing and thawing of poultry.

Factors Affecting Drip Losses

After making quantitative studies on drip, Sair and Cook (1938 a) concluded that, regardless of rate of freezing, whole birds did not drip. However, removal of skin, cutting of meat, and particularly mincing the meat increased the susceptibility of frozen-defrosted tissues to drip.

According to Reay (1934) the quantity of expressible fluid in frozen fish depended on the number of cuts made in the flesh. The major determining factor for the amount of drip in haddock was the time spent in the temperature range of -1 to -5°C (30.2 to 23°F) either during thawing or freezing.

Koonz and Ramsbottom (1939 b) reported that the freezing temperature was a factor which might have affected the amount of drip since it determined the size, distribution and number of crystals. Fast freezing favored reabsorption of the water and created a maximum fiber to water relationship in the defrosting tissue. Similarly, Callow (1952) and Empey and Howard (1954) reported that slow freezing resulted in more drip.

Pearson and Miller (1950) froze steaks at a slow rate (insulated box in walk-in freezer 0°F), an intermediate rate (chest-type freezer 0°F), and rapid rate (freezer plate -40°F) and held them for varying storage periods (0, 90, 180 days) at 0°F. They reported that the rates of freezing did not alter the expressible fluid. However, the amount of drip increased with freezer storage time. Hicks et al. (1955) in studies on the freezing, storage, and transportation of frozen meat did not find any significant influence of freezing rate on the amount of drip obtained.

Hiner et al. (1945) studied the effects of freezing temperature on drip losses in beef. The quantities of drip obtained on thawing decreased as freezing temperature was lowered from 18 to -114°F. They attributed the difference to increased intrafibrillar freezing and rupturing of fibers, which permitted the proteins to reabsorb a large proportion of the water originally frozen in the meat.

Nichols and Mackintosh (1952) investigated the structural changes which occurred in muscle tissue during repeated freezing and thawing. They concluded that muscle fibers appeared to be damaged more than connective tissue.

Chicken fryers, fowl, turkey fryers and mature turkey toms were frozen by different methods including brine, plate, and moving air (Marion and Stadelman, 1958). The method of freezing exerted no significant effect on the amount of drip. Drip from chicken fryers ranged from 5.3 to 5.8 percent; fowl 5.2 to 5.6 percent; turkey fryers 4.3 to 5.3 percent; and mature turkeys 2.3 to 3.0 percent.

Moran (1932) found that drip reached a maximum when tissues were frozen at -20°C and stored from -2 to -3°C for 80 days. He also

reported a denaturation effect at -1.5°C and at temperatures lower than -3°C.

Results obtained by Moran and Hale (1932) showed that time of storage at any one temperature had little effect on the amount of drip, but that temperature of storage made a difference. Meat was frozen in brine at -20°C, held for 24 hours at this temperature and stored at temperatures of -3.1°, -10.1° or -20.3°C. Using 10°C (50°F) for rapid thawing and 1°C (33.8°F) for slow thawing, they found no variation in drip due to thawing rate.

According to Ramsbottom and Koonz (1940) the amount of drip from steaks increased as the period of storage between slaughter and freezing increased.

Spencer et al. (1956) investigated the effects of cooling and freezing on moisture loss during thawing of turkey meat and concluded that weight loss was significantly affected by cooling treatment. Losses for carcasses not cooled in ice-water averaged about 2 percent for turkey fryers, 1.5 percent for young toms, 0.5 percent for young hens and mature hens. The ice-water cooled birds lost from 2-4 percent during thawing with the highest losses found in fryers and young tom turkeys. There was a tendency for turkeys frozen at 0°F (-17.8°C) to lose slightly more weight during thawing than those turkeys frozen at lower temperatures.

Ramsbottom and Koonz (1939) reported that irrespective of freezing temperature, there was little drip in large rib cuts of beef where the area of the cut surface was small in relation to the volume of meat. In small steaks where the area of cut surface was large in relation to the volume of meat, the amount of drip was dependent to a

larger extent on the freezing temperature. In large cuts the muscle tissue had the opportunity to reabsorb the frozen-out water; in small cuts the fluid was more readily lost from the tissue as drip.

Empey (1933) studied the effect on drip of rates of freezing and thawing, age of animal, length of time between slaughter and freezing, period in frozen state, sex, breed and composition of the muscle including pH. He found that only pH affected volume of drip; the lower the pH the greater the volume of drip collected.

According to Bouton et al. (1957) the rate of decrease in amounts of drip in psoas and l. dorsi muscles with increased pH was greater in the pH range 5.4-5.8 than at higher pH values. Beyond pH 5.8 drip values decrease gradually to a low figure. The volume of drip from l. dorsi was approximately twice that from psoas at the same pH (Howard and Laurie, 1956), indicating that important quantities of drip were exuded at somewhat higher values by l. dorsi. It was concluded that the effect of pH on fluid release from muscle protein during thawing was not identical in different muscles.

Sair and Cook (1938 b) reported that the quantity of drip obtained from meat frozen at a constant rate was affected by the pH of the tissue and the period of time between slaughter and freezing. Meat at pH 6.5 or higher did not drop in pH as a result of freezing rates which required less than 3 days to pass from $0^{o}C$ to $-5^{o}C$. At pH 5.2-5.5 the amount of drip reached a maximum, and in this region increased freezing rates reduced the amount of drip obtained. This behavior was attributed to the high water-retaining capacity of the tissue proteins at pH 6.4 which resulted in the complete retention of the water produced on thawing, regardless of the size of ice crystals

formed during freezing. At pH 5.2 the water-retaining power of the proteins was lower and moisture losses occurred. These losses were reduced by rapid freezing which produced smaller ice crystals and a more uniform distribution of water on melting. The reduced moisture-retaining capacity of tissue at pH 5.2-5.5 was due to isoelectric conditions rather than to accelerated denaturation in this region.

According to Ramsbottom and Koonz (1940) thawed beef with pH values between 6.2 and 6.5 produced only 0.7 percent drip compared with 4.3 percent drip for thawed beef of pH 5.7-5.9.

Smorodintsev and Bystrov (1937) examined the effects of freezing on the swelling of tissues in buffered solutions. They concluded that the quantity of tissue secretion varied inversely with the degree of swelling. Starting at pH 3.4 the swelling of frozen tissues increased with increased acidity of the medium and began to decrease with alkalinity (starting at pH 6.0).

According to Koonz and Ramsbottom (1939 b) ground dark chicken meat did not produce as much drip as ground white chicken meat regardless of the freezing temperature (-45.5°C, -26.1°C, -13.3°C). The pH values for meat 2-3 hours after slaughter were 5.7 for the white meat and 6.1 for dark meat. After a 22 day storage the pH values were 6.21 and 6.35 respectively. They indicated that the reabsorptive capacity exhibited by the dark muscle might have been due to the higher pH values.

Kaloyereas (1947) stated that for products with high bound-water content rapid freezing was not as beneficial for decreasing drip loss as with products of low bound-water content. A definite relationship existed between the drip of various products and their bound-water

content.

According to Hamm (1953, 1958) the amount of drip was closely related to the water-holding capacity (WHC) of meat. The higher the WHC of meat before freezing, the higher it was after defrosting. Kuprianoff (1952) reported that meat with a pH in the range of its isoelectric point (5.5-5.0) showed a greater drip loss after freezing and thawing than meat with a higher pH. At relatively high pH values (6.3-6.4) there was no drip on defrosting under certain circumstances.

Drip losses from frozen aged meat were much less than those from frozen rigor muscles (Wierbicki et al. 1957 a; Bouton et al. 1958) since the aging of meat resulted in increases in water-holding capacity (Hamm, 1960).

According to Wierbicki et al. (1954) when whole animals or quarters of beef were infused with NaCl, the water-holding capacity of the meats was markedly increased so that little or no drip occurred after freezing and thawing.

Wierbicki et al. (1957 b) studied the effects of added cations on meat shrinkage at 70°C. The cations sodium, calcium, potassium and magnesium increased to the water-holding capacity of meat. When NaCl was added to the meat prior to freezing less drip was encountered on thawing. Similar results were obtained by Empey and Howard (1954). Wierbicki (1957 b) stated that the pH shifts toward alkalinity produced by the addition of NaCl decreased after freezing and thawing. This indicated possible protein modification due to freezing. According to Whitaker (1959), pH affected the water-holding capacity of the meat by modifying the charges of the protein.

Koonz and Ramsbottom (1939 a) studied the histological structure of frozen poultry. When small pieces of meat were frozen almost instantaneously, the water frozen within the fibers appeared as minute evenly distributed ice columns. When tissues were frozen somewhat slower, fewer columns were present within the fiber, but they were large in diameter, and had a peripheral distribution. With a further rise in freezing temperature a single centrally located ice column resulted. When the freezing process was sufficiently prolonged, a temperature at which water was lost to the fibers was reached, and water froze externally to the fibers.

According to Ramsbottom and Koonz (1939) in rapidly frozen small steaks, intrafiber freezing occurred, and the drip was retained on defrosting. In steaks which were slowly frozen, extrafiber freezing took place; upon defrosting more of the fluid was lost as drip before it could be reabsorbed by the partially dehydrated fibers.

The factors which influence the size of and location of ice crystals were found similar in poultry meat to those in fish and mammalian meat (Lowe, 1948; Koonz, 1955).

Rigor Mortis and Thaw Rigor

The depletion of ATP in muscle immediately post mortem was associated with stiffening (Bate-Smith and Bendall, 1947) and shortening (Bendall, 1951) of the muscle (rigor mortis). Rough mechanical handling or freezing before the development of rigor mortis accelerated the breakdown of energy-rich nucleotides (Partmann, 1963) and hastened the onset of rigor.

Szentkiralyi (1957) reported that in the contracture that followed the freezing and thawing of cross-striated muscle of rabbit,

there was a very rapid splitting of ATP accompanied by an instantaneous deamination. Within a few minutes, all the available adenosine was converted to inosine; the final product was IMP (inosine monophosphate).

Partmann (1963) stated that when muscle tissue was frozen immediately after death, the ATP present at that moment persisted. When the temperature increased sufficiently on thawing, the ATP was split quickly under contraction of the tissue and produced thaw rigor. When the muscle contracted in thawing to about 50 percent of its former length, the condition was accompanied by a high drip loss. In whales, the drip loss amounted to about 30-40 percent of tissue weight (Sharp and Marsh, 1953; Tanaka and Tanaka, 1955). Marsh and Thompson (1957) suggested that this was the result of cell membrane rupture. Love (1962 b) reported that pre-rigor frozen fragments had about 60 percent lower soluble-protein values after thawing; he suggested that the exudation was the result of change in the proteins which reduced their ability to bind water.

According to Marsh and Thompson (1958) thaw rigor harmed protein only when contraction was allowed to take place freely. Strips of pre-rigor mammalian muscle, frozen while bound to a frame to prevent shortening, did not exude drip on thawing. Pre-rigor muscle on the animal carcass (sheep) , frozen while still attached to the skeleton, behaved in a similar manner.

ATP has been shown to decompose quite readily in frozen cod muscle maintained at $0^{o}C$. However, the pattern of nucleotide degradation at subzero temperatures differed considerably from that found in cod at $0^{o}C$ (Jones and Murray, 1961 a; 1961 b). Partmann (1963) reported that the ATP of the white muscle of carp and rainbow trout was

split at temperatures near 0°C. At -2°C it was split more quickly than in unfrozen muscle at 10°C. This activation of enzymatic ATP breakdown at the beginning of freezing was not found in the muscle tissue of chicken and beef. Within the freezing temperature range the reaction velocity of ATP breakdown started to fall quickly with decreasing temperatures. The energy rich nucleotides in beef, chicken, rainbow trout and carp muscle were split in 10-15 days at -8°C. At -24°C, 70-100 percent of the initial ATP content was normally still present after 6 months.

Tanaka and Tanaka (1958) reported that when pre-rigor frozen whale muscle was kept at -3°C for 10 days before being thawed, drip loss, exceptionally great in whale meat, could be kept low. Similarly, Marsh and Thompson (1958) found that narrow strips of pre-rigor frozen sheep muscle did not shorten or release drip when stored at -3.5°C for 4 days.

According to Howard and Lawrie (1957) two ways to reduce drip from frozen beef muscle on thawing depended on biochemical factors. Their importance has been suggested from fundamental work in vitro (Empey, 1933; Bendal and Marsh, 1951; Marsh, 1952 a, 1952 b). These factors apparently enhanced the capacity of muscle proteins to retain fluid. They were: 1) reduction in the rate of ATP breakdown during the onset of rigor mortis, and 2) restriction of postmortem production of lactic acid from glycogen which resulted in a high ultimate pH of the muscle tissue.

Methods of Collecting Drip

Various means have been devised to collect drip, some more elaborate than others. Cook et al. (1926) simply measured drip

quantitatively by using absorption by blotting paper, and thus determined loss in weight of the sample.

Hiner et al. (1945) froze beef samples overnight in an air blast at -40°F. The frozen samples were weighed and suspended over a large funnel inserted in a glass bottle and held in a temperature range of 7.2 to 8.9°C, (45-48°F). Samples remained in this temperature range approximately 24 hours to become completely thawed. Weights were obtained for the drip and the defrosted sample.

Pearson et al. (1951) reported that steaks used for drip analysis were wrapped in cellophane, overwrapped with butchers paper, frozen and stored in a home freezer at 0°F. In preparation for analysis the longissmus dorsi muscle was dissected from the remainder of the steak and thawed for 14 to 15 hours at approximately 26°C in a large funnel which drained into a graduated cylinder. The meat sample was weighed before and after defrosting, and the drip was measured to the nearest 0.1 ml.

Koonz and Ramsbottom (1939 b) collected drip from frozen molds of ground dark and white chicken meat. The molds were placed in large containers on raised wire meshing and defrosted for two days at 10°C (50°F).

A unique method for determining drip of frozen products was developed by Kaloyereas (1947) using ether or Skellysolve B, previously saturated with water, as the thawing medium. The frozen sample was placed in a beaker or other suitable container, covered with petroleum ether and allowed to stand for 24 hours. The sample was drained over a mesh screen and the volume of the aqueous phase was read directly in a graduated cylinder.

To measure the amount of fluid lost by thawed fish meat, some investigators used a method in which samples were submitted to pressure, since much of the free liquid in fish thawed after storage at relatively high temperatures was retained by simple physical forces (Banks, 1955). Banks submitted fillets to a pressure of 7 p.s.i. for 6 hours under relatively humid conditions at 0°-2°C.

Howard et al. (1960) reported on the composition of drip fluids obtained under different conditions. In the collection of drip, standard pieces of muscle measuring 6 by 3 by 1 cm (the shortest dimension along the fiber) were cut from the still frozen sirloin after removal from storage. The meat samples were placed on a glass grid inside air-tight dishes and thawed for 48 hours at 10°C. Drip fluid collected under the samples and was reported as the percentage loss in weight of the samples.

Drip Composition

Godeaux (1957) stated that because of cell membrane destruction, fluid which escaped from a muscle during thawing contained large amounts of proteins (myogen and globulin x).

Pearson et al. (1959) studied the amino acid content of drip from thawed pork. Amino acid losses were determined by microbiological assay. Losses ranged from 7.15 percent for tryptophane to 11.08 percent for isoleucine. The losses did not appear to be associated with the water solubility of the individual amino acids which indicated that the losses in drip may have been due in part to the leaching of more complex substances.

Taylor (1931) determined the percentage of protein loss and juice loss from haddock kept in storage for 32 days and defrosted over

an 18 hour period. Both protein loss and juice loss increased with time up to 62 days, after which they decreased.

Pavlovskii and Grigoreva (1963 b) studied proteolytic changes in meat during storage and upon defrosting. Electrophoretic patterns were similar for the extractable proteins of meat and the proteins in the drip. Loss of protein in the drip was 15-22 percent greater for meat subjected to preliminary autolysis before freezing than for quick-frozen meat. During the initial storage period, the proteins of myoplasmic tissue increased in the drip. After three months of storage, no increase in volume of drip was observed, and the amount of myoplasmic proteins in the drip decreased.

Seagran (1958) reported that drip in fish was due, at least in part, to the denaturation of protein which normally tended to hold the water of the muscle. By dilution techniques and electrophoresis, he showed a definite similarity between the protein composition of drip and extracts of low ionic strength from fish muscle. He found the contractile protein, actomyosin, absent in drip from frozen and thawed rockfish. He concluded that the sarcoplasmic fraction of fish muscle was not intimately associated with the origin of drip, and suggested that drip formation and texture changes,which resulted from freezing and thawing, were due in part to actomyosin denaturation by a dehydration process.

Howard et al. (1960) reported on the composition of drip and possible origin of solutes in drip fluid. A higher concentration of both hemoglobin and myoglobin was reported in the drip than in whole muscle. The higher hemoglobin presumably arose from the residual fluid in the vascular system. This hemoglobin containing fluid moved

more readily through the sample than fluid contained within the cells and interstitial spaces. A similar increase in concentration of myoglobin was expected when the pigment was confined to a portion of the cell fluid which moved more readily than that associated with the fibrillar protein.

Howard et al. also reported that the proportion of chloride in the ash of drip was approximately 4 percent which indicated that about 8 percent of the ash constituents were present in vivo in the extracellular space. This also indicated fairly complete mixing of the extra- and intra-cellular fluids. It was concluded that laboratory drip did not represent the discharge of interstitial fluid in vivo, but was, regarding inorganic components, mostly of intracellular origin. The intracellular components were discharged either through cut ends of cells or through altered cell walls. The conclusion that the soluble constituents of drip were mainly of intracellular origin was in agreement with the work of Seagran (1958) on cod muscle, and also with the conclusions of Connell (1957) for expressible fluid from fish fillets.

Paper electrophoresis and ultracentrifuge results (Howard et al. 1960) indicated that drip had patterns similar to those from extracts of muscle of low ionic strength. While freezing and thawing modified the solids content of the drip fluid, they did not modify the composition of these solids. Modifications brought about by freezing were largely restricted to structural proteins which were insoluble at the ionic strength of the drip fluids.

Freezing and Thawing of Meat

According to Taylor (1930) certain physico-chemical changes

in the frozen state, rather than the rupturing of cells by expansion or puncturing of cell walls by ice crystals, provided the basic explanation of drip obtained from thawed fish and meat. He stated that the behavior of frozen foods with respect to fluid loss, was related to colloidal protein systems and the conditions under which they were converted from liquid to gel or gel to liquid, coagulated or separated into heterogeneous systems. Taylor suggested that the three means by which the cell jelly could have been converted to a free flowing liquid were by: 1) the salting out of protein by strong brine in the frozen cells, 2) the contraction of the jelly and squeezing out of the then dilute liquid, and 3) autolysis of the cell membrane. The cell membrane would have remained undamaged by quick-freezing, and the juice would have been unable to escape through the membrane. The process of autolysis offered a possible explanation since it might account for digestion of the cell membrane or increased cell membrane permeability.

Riddell *et al.* (1927) reported on denaturation of protein in fish muscle and fish muscle juice. The extent of denaturation was governed largely by storage temperature and length of holding period. Reay (1933, 1934) drew attention to the denaturation phenomenon after he investigated the influence of freezing temperatures on haddock. He found that the solubility of muscle proteins in salt solutions decreased after frozen storage of the fish. The globulin (actomyosin) fraction was affected the most and change was greatest in the temperature range of -2 to -6°C. He concluded that the denaturation and deterioration which occurred during the thawing process was similar to that which occurred during freezing. Nikkila and Linko (1954) also

reported a rapid protein denaturation during the thawing of frozen fish.

Plank (1934) pointed out the importance of two effects of freezing: 1) the damage to the protoplasmic structure by formation of ice crystals, and 2) the consequent dehydration of the colloid. Luijpen (1957) reported that the denaturation phenomena in frozen fish was a result of the action of concentrated salt solutions on the soluble proteins.

Dyer (1951) stated that the formation of ice crystal fragments in frozen fish was attributed to the dehydration of tissue cells. The proteins present in the sol form in the fresh tissue were converted to gel form at the eutectic point by denaturation of the salts in the fresh tissue.

Finn (1932) stated that rapid freezing and thawing together with storage at low temperatures was important in denaturation, since these procedures minimized changes due to denaturation of the proteins by strong salt solutions. Smorodintsev and Bystrov (1937), and Drozdov and Drozdov (1939) reported that freezing at low temperatures (-25° to 60°C) resulted in no denaturation of muscle proteins as determined by ATP-ase activity or the solubility and swelling of tissues in salt solutions.

Deatherage and Hamm (1960) found that quick freezing and thawing caused no significant decrease in hydration and no change in either isoelectric point or pH. Changes in acidic and basic groups of muscle proteins by quick freezing were small but significant.

In the field of red meats, Bystrov (1938), Artyukh (1940), and Smorodintsev (1943 a, 1943 b) have reported that there were no

extensive changes in the proteins during frozen storage, and modifications which did occur were of little significance. However, several investigators have provided evidence of some autolytic activity in frozen beef and pork (Foster, 1924; Hiner et al., 1951; Shrewsbury et al., 1942).

Love (1955 a) reported that while the overall extent of denaturation depended on length of storage, his results showed clearly that large differences in denaturation existed between fish frozen at different rates. Therefore, an important factor affecting denaturation was the mode of ice formation, distribution, and the resulting concentration patterns of tissue parts rather than actual mechanical damage (Love, 1956).

After re-examining the question of tissue damage, Love (1955 a) reported that the appearance of deoxypentosenucleic acid (DNA) in the muscle expressible fluids indicated a rupture of the sarcolema and a liberation of nucleic materials. The presence of DNA provided a means of assessing the degree of cellular damage. As fish fillets were frozen more rapidly, the expressible nucleic acid suddenly rose to a maximum; this was thought to correspond with the point of formation of intracellular ice crystals. In ultra-rapid freezing there was also a rise in expressible DNA which was probably due to a different kind of cell damage.

Love (1958 a) further examined the action of intercellular ice on muscle cells by measuring DNA (Method of Love, 1955 a) in fish fillets frozen at different speeds (solid CO_2 and plate at -78°C. He found a zone of minimum damage at a freezing time of about 115 minutes, the most rapid freezing at which all ice was able to form in the inter-

cellular spaces. Since the ice masses were relatively small (little larger than the muscle cells) they did not seriously deform or damage the cells. Cell damage increased during slower freezing to a maximum value at 200-500 minutes and then decreased. During very slow freezing, the strong solutions, which resulted from the freezing out of water from the weakly saline interstitial fluids, exercised a considerable solvent effect on the fibers without actually rupturing them. The concentration of protein in the interstitial fluid of slowly frozen fish (about 750 minutes) tended to increase.

Since a lower amount of ice resulted in a decreased concentration of tissue salts, less denaturation could have occurred when the quantity of ice present at a given temperature was reduced (Love, 1962 a). These results supported the contention (Love 1958 b) that denaturation was caused directly or indirectly by the action of concentrated salts on the proteins.

Vickery (1926) emphasized the importance of the effect of freezing beef and mutton on the sarcolema. He indicated that the sarcolema had an important role in determining the amount of reconstitution which took place. Sherill (1928) showed that slowly frozen haddock contained damaged muscle structure, especially ruptured sarcolema.

Khan et al. (1963) reported that chicken meat muscle proteins were denatured and proteolysis occurred during frozen storage. Decreased ATP-ase activity and protein solubility was attributed to a stepwise denaturation of actomyosin. Proteolysis occurred in both breast and leg muscle at -18, -10 and -4°C. The rate of change was directly related to storage temperature and time. Muscle proteases were

found to have pH optima of 4 and 7 and temperature optimum of 37°C (Bandack-Yuri and Rose, 1961; Slinwinski et al. 1959). The pH of muscle was found to vary from 5 to 7 (Bate-Smith, 1948), and he reported that enzyme activity should have been influenced by both pH and temperature. However, Balls (1938) reported that beef contained a protease active at freezing temperatures, and Khan et al. (1963) concluded that a similar enzyme system was probably present in chicken muscle.

After studying the denaturation of proteins in frozen fish, Sawant and Magar (1961) concluded that denaturation was restricted to the actomyosin fraction of protein while the sarcoplasmic fraction remained unchanged. This was essentially in agreement with Dyer's (1951) observation that albumen fraction was not denatured during frozen storage. Similarly, the findings of Seagran (1958, 1959) suggested that the sarcoplasmic fraction was not associated with the formation of drip.

Connell (1960) determined the ATP-ase activity and easily-reactable and total sulfhydryl groups of cod flesh during frozen storage up to 170 weeks at -14°, -22°, and -19°C. At -19° no change in enzyme activity was detected, but at -22° and -14° definite loss of activity occurred. No change in easily-reactable or total sulfhydryl groups was detected at any temperature. These findings are in accord with those of Partmann (1954). Khan et al. (1963), however, reported that the number of sulfhydryl groups in chicken meat decreased during frozen storage.

Pavlovskii (1962) studied autolytic and denaturation changes in muscle tissue during cooling and freezing of meat. He stated that

autolysis of muscle was characterized by degradation, which resulted from ATP-ase activity, glycolytic conversion and resynthesis of adenoise triphosphate (ATP). Freezing increased NaCl, myosin and ATP-ase concentrations. He concluded that the denaturation of frozen muscle was characteristic of meat previously autolyzed before freezing.

Massi (1958) reported that no amino acid increases took place in frozen meat. However, proteolysis occurred very rapidly during and after defrosting leading to the destruction of the molecular structure.

After a study of quick frozen meats (veal and beef) Monzini (1953 a) reported that ammonia nitrogen remained low (as in fresh meat), but amino nitrogen increased to 5 to 6 percent of the total nitrogen; this was considerably higher than the minute quantities present in fresh meat.

Swanson and Sloan (1953) reported that proteolysis in poultry was indicated during frozen storage by an increase in soluble nitrogen and non-protein nitrogen of both leg and breast meat. Decreases in amino acid nitrogen suggested that certain metabolic processes continued in the frozen state and resulted in an additional breakdown of amino acids which were formed during proteolysis.

Pavlovskii and Grigoreva (1963 a) studied the protein components of autolyzing muscle tissue during cooling and freezing of meat. Cooling and freezing decreased the amount of readily extractable protein of the tissues. During initial storage of frozen meat the soluble protein increased, but after three months this fraction decreased.

According to Monzini (1953 b) the catheptic and tryptic activities of the enzymatic extracts of beef increased after freezing.

Intensity of enzymatic activities were increased by repeated freezing and thawing but were not affected by the freezing temperature. During 48 months of storage reduction in amino nitrogen and an increase in ammonia was noted.

Microbiological Assay for Amino Acids

Microbiological assays have been developed for accurate measurements of the amino acid composition of foods and purified proteins. These developments have been summarized by a number of reviewers (Snell, 1944; Block, 1945; Schweigert and Snell, 1947).

The amino acid requirements of L. mesenteroides for a given microbiological procedure for quantitative determinations of an amino acid were reported by Dunn et al. (1944). These requirements were:

"1. The amino acid must be essential for the growth of the organism.

2. The basal medium should contain the minimum concentration of nutrients required to produce a standard curve of maximum slope.

3. The standard curve should be reproducible within close limits and approximately linear over the segment to be employed for analytical purposes.

4. The assay should be applicable over a 4-fold and preferably greater range of concentration."

The amino acid requirements of S. faecalis were investigated by Greenhut et al. (1946). Leucine, threonine, glutamic acid, asparagine, lysine, isoleucine, methionine, arginine, valine, histidine, serine, tryptophane and cystine were required for growth while alanine, tyrosine and phenylalanine stimulated growth.

Steele et al. (1949) reported on a medium for the microbiological assay of amino acids based on the amino acid requirements for growth of L. mesenteroides P-60. The d-forms of most amino acids were inactive for L. mesenteroides.

According to Hegsted (1944) the single omission of arginine, cystine, glutamic acid, isoleucine, methionine, phenylalanine, tyrosine, leucine or valine from a medium containing 19 amino acids prevented growth of L. arabinosus. However, threonine, aspartic acid and lysine were required for growth in addition to the previously mentioned 10 amino acids.

Kuiken et al. (1943) reported a microbiological assay procedure for the determination of leucine, valine and isoleucine in food stuffs. Methods of preparing standard solutions of the amino acids were described. Purified proteins were hydrolyzed by refluxing for 24 hours with 5N sulfuric acid (about 40 ml per gm of protein), and hydrolyzates were neutralized with hot saturated barium hydroxide solution. Only the naturally occurring optical isomers of the three amino acids were active. The presence of carbohydrates during hydrolysis of the proteins resulted in increased formation of humin with a corresponding loss of protein nitrogen. Loss of the three amino acids due to humin formation was relatively small.

Hier et al (1945) assayed ten amino acids in casein and beef muscle, and stated that autoclaving time was very important, as excessive autoclaving resulted in low maximum growth and unsatisfactory assays. This was especially true for threonine. Variability in results, when calculated as micrograms of amino acids per cc, was well within a 10 percent range and usually within 5 percent. After 72 hours

of incubation there was no indication of drift in values. This is essentially in agreement with the work of McMahan and Snell (1944) who reported that after a 3 day incubation period, there was no tendency for values to drift upward or downward. During a 24 hour incubation period, growth did not proceed to completion and drifted upward or downward at increasing dosage levels.

Schweigert et al. (1944) determined the valine and leucine contents of fresh animal tissue. Satisfactory hydrolysis of animal tissues was obtained by autoclaving one (1) gram samples with 2N hydrochloric acid for 5-10 hours at 15 pounds pressure. Other effective methods of hydrolysis were refluxing for 24 hours with 2 or 4N hydrochloric acid and refluxing for 24 hours with 5N sulfuric acid. Treatment with hydrochloric acid provided a more rapid hydrolysis than with sulfuric acid at the same normality. Results were calculated on the basis of 100 percent activity for the l-isomers; 50 percent activity for the dl-isomers and no activity for the d-isomer.

Greenwood et al. (1951) determined the amounts of 18 amino acids in the protein of different cuts and grades of beef. The amounts of amino acids were similar in all cuts and grades tested. The percentage of total nitrogen accounted for by the 18 amino acids in choice and utility beef was 87.6 percent. This figure is quite similar to the values of 85.1 and 84.9 percent obtained for lamb and pork cuts (Schweigert et al., 1951).

Horn et al. (1949) reported the use of L. mesenteroides in the determination of leucine in 31 proteins and foods. Results agreed closely with those obtained on the same materials using other microbiological methods.

According to Lyman et al. (1946 a) one of the most useful methods for testing the reliability of assay values obtained by microbiological assay was to carry out the test with more than one organism. They determined the methionine content of foodstuffs colorimetrically and by microbiological assays using L. mesenteroides and S. faecalis-R as the test organisms. All three methods gave similar values. Proteins and foodstuffs were hydrolyzed by refluxing 0.5 to 2.0 gram samples with 100 ml of 6N hydrochloric acid for 24 hours. Refluxing for 24 hours with 6N sulfuric acid and autoclaving at 15 pounds pressure for 6-8 hours with 3N hydrochloric acid also gave values which were in excellent agreement.

Riesen et al. (1946) came to similar conclusions using L. arabinosus as well as S. faecalis and L. mesenteroides in assays for methionine in proteins and foodstuffs. They stated that the maximum growth obtained with L. arabinosus was greater than with the other two organisms. Since no destruction of methionine was observed on prolonged hydrolysis, a 10 hour hydrolysis (autoclaving with 5N hydrochloric acid) was preferred to 5 hours.

Schweigert et al. (1949) hydrolyzed samples of pork and lamb cuts by autoclaving 0.5 gram samples in 25 ml of 3N hydrochloric acid for 16 hours at 15 pounds pressure. Results obtained for methionine by chemical methods and microbiological techniques were similar for samples varying in methionine content. Excellent agreement was obtained in the assay for histidine with either S. faecalis or L. mesenteroides was used as the test organism or when phenylalanine was assayed with either L. arabinosus or L. mesenteroides.

Sirny et al. (1950) reported on the hydrolysis of meat for arginine and histidine determinations. Hydrolyzates were prepared by autoclaving five gram samples of meat (containing approximately one (1) gram of crude protein) with 40 ml of 2N hydrochloric acid for 5 hours at 121°C. in covered Erlenmeyer flasks. The filtered and diluted hydrolyzates were stored at 5°C until amino acid analyses were made.

Fry and Stadelman (1960) studied the optimum conditions of hydrolysis for the microbiological assay of methionine and cystine in poultry meats. One gram samples of ground, moisture-free, fat-free meat were hydrolyzed with 10 ml of 3N hydrochloric acid under reflux or in sealed tubes at 15 pounds pressure. Methionine values determined turbidmetrically were somewhat lower than values obtained acidmetrically. Turbidimetric readings were difficult to make at the higher levels of methionine as the growth curves failed to maintain a straight line when plotted on log-log paper. Turbidimetric determinations of cystine were even more difficult. Sealed tube hydrolysis with 3N hydrochloric acid required a longer time than refluxing with 6N hydrochloric acid; however, with the sealed tube less destruction of the amino acids was obtained after maximum liberation of the amino acids. A sealed tube hydrolysis period of 8 hours was recommended for methionine, and a 4 hour sealed tube hydrolysis was recommended for cystine.

Greene and Black (1944) reported the possible use of a pancreatic preparation to hydrolyze food and protein samples in microbiological assays for tryptophane. Complete racemization took place under alkaline hydrolysis so that the observed values were multiplied by 2.

Schweigert et al. (1945) studied the extent of hydrolysis

of protein in animal tissues by acid, alkaline and enzymatic treatments. They found that when a preparation of intestinal mucosa from rat and hog intestines was added to a digestion mixture with pancreatin, alpha amino nitrogen and leucine, the tryptophane values were higher than values obtained with pancreatin digestion alone. The alpha amino nitrogen obtained by using the pancreatin and intestinal mucosa was approximately equal to that obtained by acid hydrolysis.

Stokes et al. (1945) determined the tryptophane content of protein by hydrolyzing 0.5 gram samples with 10 cc of 5N sodium hydroxide in Erlenmeyer flasks plugged with non-absorbent cotton, at 15 pounds pressure for 10 hours. Purified proteins were hydrolyzed in sealed tubes (25 mg protein with 2 cc of alkali). A copious precipitate frequently appeared after neutralization of the digestion mixture. This was believed to contain considerable quantities of silica dissolved from the flask. The precipitate was removed by centrifugation. Alkaline hydrolysis used to liberate tryptophane from protein resulted in complete racemization of the amino acid. The d-form was inactive for S. faecalis; therefore, the assay values obtained were multiplied by 2 to arrive at the final correct tryptophane content. For acid hydrolysis one (1) gram of protein was autoclaved with 10 cc of 10 per-cent hydrochloric acid in sealed ampules for 10 hours at 15 pounds pressure.

Kuiken et al. (1947 a) stated that the destruction of trypto-phane, which occurred during hydrolysis with sodium hydroxide, was due to oxidation by molecular oxygen. The use of l-cysteine as a stabilizing agent was described. Tests with the Bancroft-Warburg apparatus showed that tryptophane reacted with molecular oxygen in an alkaline solution

at room temperature. Catalytic effects of copper, nickel and platinum were observed. It was suggested that cysteine may have acted by removing oxygen from solution or by combining with copper or other heavy metals in order to block their catalytic action.

Muscle Composition

The essential amino acid composition of meat and 30 other foods was determined by Lyman and Kuiken (1949), by means of microbiological assay procedures. Nineteen (19) different kinds of meat were examined including tissues from beef, pork and lamb. Very little variation was noted in the amino acid content of similar samples taken from different animals, whether the source was beef, pork or lamb. However, wide variations were found in the histidine content of muscle tissue.

Orr and Watt (1957) reviewed the findings of many laboratories and presented data for the 18 most frequently occurring amino acids of foods. Results were expressed both as grams of amino acid per gram of total nitrogen and in terms of average content of amino acid per 100 grams edible protein.

Beach et al. (1943) studied the amino acid composition of the protein mixtures of ten edible muscle meats including chicken, beef, veal, shrimp, pork, salmon, codfish and froglegs as well as six beef organ tissues (liver, kidney, heart, brain, stomach and lungs). The ten essential amino acids in the protein mixture from voluntary muscle tissue were found to be similar in Aves, Mammalia, Amphibia, Pisces, and Crustacea. The muscle tissues of these various classes of animals did not differ widely in their amino acid composition. This supported the theory that similar amino acid composition of muscle proteins was

repeated throughout the animal kingdom. Certain differences in amino acid composition were found among the muscle proteins of different species. These were attributed to the higher concentration of nuclear materials and different functional activities in the various species.

Millares and Fellers (1948, 1949) emphasized the absence of available literature on determinations of amino acids in chicken meat prior to 1948. Only eight essential amino acids and two non-essential amino acids had been determined. They reported that chicken meat was an excellent source of the essential amino acidsand that chicken meat was equivalent to beef, pork, lamb and veal on the basis of content of these amino acids.

Koonz and Robinson (1946) studied the histological and chemical composition of 12 of the more important muscles which collectively made up approximately 70 percent of the muscle tissue of the poultry carcass. White muscles had relatively little fat, were low in moisture and high in protein. The pH of the white muscle was lower than that of the dark, and after initial post mortem drop, there was little change in pH over a 48 hour period.

Similar differences in pH values between dark and white chicken meat were previously reported by Koonz and Ramsbottom (1939 b). Initial pH values (2-3 hours after slaughter) were 5.7 for white meat and 6.1 for dark meat. After a 22 day period the pH values were 6.35 and 6.21 respectively.

Strandine et al. (1949) reported variations in pH, protein, fat and moisture content of beef and chicken muscle. The pH of chicken muscle measured 48 hours after slaughter ranged from 5.6 for the pectoralis superficalis of the breast to 6.0 for the adductor of the

thigh. Fat content ranged from 1.3 percent in the pectoralis profundus muscle to 10.3 percent in the adductor muscle. The moisture content varied from 69.6 percent in the adductor to 75.1 percent in the biceps femors. Protein content ranged from 19.9 percent to 25.0 percent for the semitendinosus and pectoralis profundus muscles respectively.

According to Scott (1959) the amino acid composition of turkey meat was similar to that of chicken, beef and pork when the amino acids were expressed as percentages of the proteins in these meats. Methionine and cystine values differed more than values for other amino acids. The sum of these two sulfur containing amino acids was lower in pork than in the other species. The amino acid composition of breast and leg meat from both male and female turkeys was constant when amino acids were expressed as percentages of protein in these tissues. However, histidine was the only amino acid found in higher concentrations in the protein of breast meat than in leg meat.

Hepburn (1950) found that broiler white and dark meat contained 20.84 and 19.27 percent protein respectively. The white and dark meat from roasters contained 21.76 and 20.15 percent protein respectively. The percentage of amino acid and basic nitrogen in broilers was 1.02 percent for light meat and 0.82 percent for dark meat. In roasters the values were 1.22 and 0.90 percent respectively.

The tryptophane content of 56 samples of meat was determined by a microbiological procedure with S. faecalis (Kuiken et al., 1947). For similar types of tissue the tryptophane content was similar for beef, pork and lamb. Variations in the tryptophane content of similar tissue taken from different animals were very small.

Lyman (1946 b) reported on the methionine content of 56 samples of meat in which L. mesenteroides was used as the test organism for microbiological assays. The methionine content of a given type of tissue was similar in beef, pork and sheep. Methionine content varied in organs such as the liver, kidney and brain.

Szkutnik (1958) performed studies of the amino acid composition of trypsinized and acid protein hydrolyzates of cattle, sheep, swine and horse meat by paper chromatography. The qualitative and quantitative amino acid composition of all the meats after 24 hours of tryptic or acid digestion was approximately the same.

PROCEDURE

This investigation was conducted in three parts. The first part included two experiments which were designed to determine the optimum methods of hydrolysis (maximum liberation of amino acids) of chicken meat and chicken drip as measured by microbiological assay. The second phase of this study was concerned with the quantitative determination of specific amino acids in chicken meat and drip held in storage for specific periods of time by microbiological assay. In the third phase, the amino acids found in chicken drip hydrolyzates were evaluated by an amino acid analyzer.

General Methods Used

Nitrogen Determination

All nitrogen values were determined using the micro-Kjeldahl method as outlined by the American Instrument Company (1961), unless otherwise stated. Nitrogen contents were reported as mg of protein nitrogen per ml of solution or per g of tissue. All nitrogen determinations were run in triplicate.

Reagents

Reagent grade chemicals and deionized water were used throughout this study. Details of standard solution composition are contained in Appendix A.

Centrifugation

A Model C S International Centrifuge was used throughout this study.

pH Measurements

All pH measurements were made with a Beckman Zerometic pH meter and observed values were recorded to the nearest one-tenth unit.

Part I

Experimental Animals

All of the chickens used in this study were heavy hens from a Cornish- White Rock cross, raised on identical rations on the Michigan State University Poultry Farm.

Twelve birds were bled, scalded in a Rotomatic scalder and feathers removed in an automatic rubber fingered picker. All of the birds were then eviscerated, washed and placed in slush ice for 18 hours. At the end of the chilling period, six birds (Group I) were drained, packaged in Cryovac bags under partial vacuum, frozen at 0°F and stored at 0°F until analyzed.

The six birds in Group II were removed from the slush ice and drained. Each bird was quickly sectioned by removing the thighs, drum sticks and wings. The Pectoralis major and minor muscles were dissected from the birds and packaged in 4 X 6 inch Cryovac bags under partial vacuum. Thighs and drum sticks were deboned, and the dark meat was packaged in a similar manner. All of the white and dark meat samples were tagged individually for identification, stacked on metal racks in a cabinet freezer and frozen at 0°F. The packages were rotated during the freezing period to facilitate a more equal rate of freezing of the meat. All samples were held at 0°F for subsequent analyses.

Sample Preparation for Microbiological Assay

Two birds from Group I were thawed in the Cryovac bags at approximately 60°F. After thawing, the white meat (Pectoralis major and minor) was dissected from each carcass, and the thighs and drum sticks were deboned (dark meat).

Each white and dark meat sample was ground twice through a 1/8 inch plate. The ground samples were collected in aluminum foil trays and spread to a thickness of about one (1) cm. The samples were covered with Saran wrap to prevent evaporation and frozen at -30°F.

To facilitate moisture removal, the Saran wrap was removed, and the frozen samples were loosened from the aluminum trays. The frozen material was placed in a Stokes-Freeze Dryer, Model 2003-F2 (F. J. Stokes Machine Company, Philadelphia). During drying, the absolute pressure in the drying chamber was maintained at approximately 0.15 mm of mercury. The condenser shelves were maintained at about -35°C and the heating shelves at 35°C. The freeze-dried samples were removed after 24 hours, placed in plastic containers, sealed and held at 0°F for analysis.

Moisture Determination

Moisture was determined by placing 10 g of chicken tissue in an aluminum dish (60 mm diameter X 15 mm) and drying at 100-102°C for 24 hours. The dishes were cooled in a desiccator and then weighed. The loss in weight was reported as moisture (Minor, 1964).

Ether Extraction

The freeze-dried samples were weighed into extraction thimbles. Complete extraction of the fat from the tissue with petroleum ether required 16 hours in a Soxhlet extractor. The extracted tissue was dried at 100°C for 30 minutes, cooled in a desiccator and weighed (A.O.A.C., 1960).

Total Nitrogen

The moisture-free, fat-free samples were pulverized with a mortar and pestle; 300 mg samples were analyzed for nitrogen by the

micro-Kjeldahl method.

Methods of Hydrolysis

The pulverized samples were hydrolyzed by one of three methods: autoclaving in a sealed tube, autoclaving in a covered Erlenmeyer flask or refluxing. The timesof hydrolysis were 1, 2, 4, 8, 16, and 24 hours. For each combination of time and method, two replicates from each of two samples were hydrolyzed.

1. Autoclaving in a Sealed Tube

The method described by Fry and Stadelman (1960) was used. Samples were hydrolyzed in borosilicate tubes 18 mm in diameter and originally 16 inches in length. The tubes became progressively shorter with use and were discarded when the sample and acid filled more than one-third of the tube. One gram of the sample was placed in each tube; 10 ml of 3N hydrochloric acid was added. The tubes were sealed, cooled, shaken vigorously and placed in racks in an autoclave maintained at 15 pounds pressure for the specified times.

2. Autoclaving in a Flask

One g of the pulverized sample was placed in each 125 ml Erlenmeyer flask, and 25 ml of 2N hydrochloric acid was added to each flask. The Erlenmeyer flasks were covered with small beakers. These were placed in an autoclave at 15 pounds pressure for the specified times.

3. Refluxing

Each one gram sample to be refluxed was placed in a round bottom boiling flask with 25 ml of 6N hydrochloric acid and connected to a condenser. Each flask was heated in a heating mantle with a Powerstat setting of 70. Refluxing was timed from the fall of the first

drop of condensate.

Preparation of Hydrolyzates for Assay

The hydrolyzates were rinsed from the containers and raised to a pH of 4.0 with sodium hydroxide solution. Approximately one-half g of activated charcoal was added; the solution was mixed and filtered. The residue was washed with distilled water, and the washings were added to the previously obtained filtrate. The pH of the combined filtrates was adjusted to 6.8 with sodium hydroxide solution. The volume was made up to 250 ml with distilled water. The hydrolyzates were frozen at -20°F in 4 ounce polyethylene bottles and held at 0°F for analysis.

Microbiological Assay

Arginine, leucine, and methionine were determined with Leuconostoc mesenteroides P-60, ATCC #8042 as the test organism (Steele et al., 1949). A stock culture of the test organism was obtained from the American Type Culture Collection, 2029 M Street, N.W., Washington, D. C.

Stock cultures were prepared by stab inoculation of Bacto-Micro Assay Culture Agar obtained from the Difco Laboratories, Inc., Detroit 1, Michigan. The culture tubes were incubated for 24-48 hours at 35-37°C and stored in a refrigerator (2-6°C). Subcultures were made in triplicate at seven day intervals.

The inoculum for assay was prepared by subculturing from a stock culture to 10 ml of Bacto-Micro Inoculum Broth (Difco Laboratories). After 24 hours of incubation at 37°C, the cells were centrifuged (1500 r.p.m., 15 min) and the supernatant liquid decanted. The cells were resuspended in 10 ml of sterile 0.85 percent saline to wash out residual

medium, recentrifuged and suspended again in sterile saline. The cell suspension was diluted 5-100 with sterile saline. One drop of this suspension was used as inoculum for each assay tube.

Standard solutions of arginine, leucine, and methionine were prepared using the l-isomers of each amino acid (Difco, 1953). Triplicate tubes containing 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 5.0 ml of standard solutions were used to construct the acidimetric standard curves.

All media were obtained from Difco Laboratories, Inc. Each medium contained all of the nutrients necessary for growth of the test organism except the one essential ingredient for which the medium was recommended. The growth response of a particular test organism was measured acidimetrically after the addition of this nutrient in specific increasing concentrations. Five ml of the rehydrated media were placed in each culture tube with additional standard solution or test sample and distilled water to make up a final volume of 10 ml. The tubes were autoclaved for 10 minutes at 15 pounds pressure, inoculated with one drop of the 5-100 cell suspension and incubated at 30°C.

After 72 hours of incubation, the tubes were autoclaved for 15 minutes at 15 pounds pressure, cooled and titrated with 0.1N sodium hydroxide. The solutions were stirred with a magnetic stirrer while being titrated to an end point of pH 7.0 as measured by a Beckman Zeromatic pH meter. Micrograms of amino acid per ml of hydrolyzates were calculated by reference to the standard curves. Grams of amino acid per 100 grams of protein were subsequently calculated, (See Appendix B).

Drip Collection

The method for collecting drip was similar to that reported by Pearson et al. (1959). The apparatus consisted of a glass funnel supported by a ring stand over a graduated cylinder. A wire grid rested on the inside of the glass funnel. The entire apparatus was placed in a 50 pound lard can which had a layer of water on the bottom to provide a saturated atmosphere.

The white and dark meat samples previously frozen in Cryovac bags (Group II) were used in this part of the study. After the packaging material was removed, each frozen meat sample was weighed and placed on the wire grid inside the funnel. A few drops of toluene were added to the graduated cylinder to reduce microbial spoilage. The can lid was placed on the can and sealed with masking tape. The entire apparatus with sample was placed in a cooler at approximately 60°F for a thawing period of about 18 hours. At the end of the thawing period, each meat sample was reweighed and the drip sample was measured to the nearest 0.1 ml. The drip was held at 2-6°C for analysis. Total nitrogen was determined by micro-Kjeldahl method using a one ml sample of drip.

Drip Hydrolysis

The optimum hydrolysis procedure for maximum liberation of amino acids from meat (determined previously) was refluxing with 6N hydrochloric acid for 16 hours. In this part of the study, the effects of a 16 hour hydrolysis on the liberation of amino acids from drip were determined.

Each five ml sample of drip was placed in a round bottom flask with 5 ml of 12N hydrochloric acid. Two samples were hydrolyzed by refluxing for each of the specified times (2, 4, 8, 16 hours).

Hydrolyzates were mixed with activated charcoal, clarified by filtration, and adjusted to a pH of 6.8. The filtrates were diluted to 250 ml with distilled water, frozen in 4 ounce polyethylene bottles at -20°F and held at 0°F for analysis.

The microbiological assay for arginine, leucine and methionine in drip was similar to the analysis previously described for meat hydrolyzates. Quantitative determinations of the amino acids were made by referring to standard curves which were constructed at the same time as the assay.

Salt Effect

After samples were treated with 6N hydrochloric acid and hydrolyzed, the digestion mixture was neutralized with sodium hydroxide. It was necessary to determine whether the sodium chloride formed would act as a stimulant or an inhibitor of L. mesenteroides.

The maximum amounts of sodium chloride present in the assay tubes for the three amino acids were : leucine 1.40 percent; methionine 3.51 percent; and arginine 7.02 percent.

Standard curves were constructed with and without the addition of sodium chloride to determine the possible effects of salt on growth. Two replicates of the standard with sodium chloride added were determined through a 10-fold range of salt concentration using L. mesenteroides as the test organism. The concentration of salt used for the three amino acids ranged as follows: arginine 0.70-7.00 percent; leucine 0.14-1.40 percent; and methionine 0.35-3.50 percent. Standard solutions of 100 ml each were mixed from the stock solution, and sodium chloride was added.

Part II

Experimental Animals

The twelve heavy hens used in this part of the study were from a Cornish-White Rock cross strain raised under similar conditions as the birds in Part I. Six birds (Group III) were slaughtered, eviscerated, and chilled by the methods used in Part I. The birds were tagged for identification, frozen in Cryovac bags at 0°F and held for analysis at 0°F. The six birds in Group IV were dissected. The white and dark meat samples were packaged, tagged and frozen in a cabinet freezer at 0°F and held at 0°F for analysis.

Three birds from Groups III and IV were thawed and analyzed 30 days after slaughter; the remaining three birds in each group were stored for 90 days at 0°F prior to analysis. At the end of the storage period the samples were prepared for hydrolysis as previously described. The tissue from the birds in Group III was freeze-dried, ether-extracted and stored in sealed containers at 0°F for hydrolysis. Drip was gathered from the frozen dark and white meat samples (Group IV) by the methods used in Part I. Total nitrogen was determined on both meat and drip samples by the micro-Kjeldahl method.

Drip Hydrolysis

Drip samples were hydrolyzed (refluxing with 6N hydrochloric acid for 16 hours) shortly after collection to reduce the possibility of microbial deterioration. The acid hydrolyzates were neutralized, diluted to convenient volumes, frozen in bottles at -20°F and held for analysis at 0°F.

Alkaline hydrolysis of the drip samples for the determination of tryptophane was carried out with sodium hydroxide in the presence of cysteine as recommended by Kuiken et al. (1947 b). The method described by Lyman and Kuiken (1949) was used with some modification. In this procedure 100 mg of l-cysteine were added to 10 ml of 5N sodium hydroxide (Scott, 1949) in a 125 ml Erlenmeyer flask. This was covered with a small beaker. The solution was autoclaved for approximately one hour at 15 pounds pressure. Another 100 mg of l-cysteine was added along with 4 ml of the drip sample to the hot solution. The samples were autoclaved for 16 hours at 15 pounds pressure, diluted with distilled water to approximately 100 ml and neutralized with standard acid solution.

In most instances a precipitate formed during neutralization, and this precipitate was removed by centrifugation. The supernatant was decanted, and the precipitate was washed with distilled water and recentrifuged. Supernatants were combined, diluted to a convenient volume, and frozen in 4 ounce bottles at -20°F. The frozen hydrolyzates were held for analysis at 0°F.

Meat Hydrolysis

Moisture-free, fat-free, dark and white meat samples (held for 30 and 90 day storage periods) were hydrolyzed by refluxing with 6N hydrochloric acid for 16 hours. The acid hydrolyzates were neutralized, diluted to a desirable volume, frozen in polyethylene bottles at -20°F and held at 0°F for analysis.

The procedure for alkaline hydrolysis of meat was similar to that previously described for the alkaline hydrolysis of drip. Meat samples weighing approximately 0.2 g were hydrolyzed with 15 ml of 5N

sodium hydroxide for 16 hours. Stokes et al. (1945) recommended a ratio of 25 mg of protein to 2 ml of 5N sodium hydroxide for alkaline hydrolysis for tryptophane determinations.

Alkaline meat hydrolyzates were diluted, neutralized, centrifuged, frozen and stored in the same manner as were the drip hydrolyzates.

Microbiological Assay of Drip and Meat

Arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine and valine were determined using Leuconostoc mesenteroides P-60 (Steele et al. 1949; Difco, 1953). Streptococcus faecalis (ATCC #8043) was used for determination of threonine as described by Steele et al. (1949) and Difco (1953). The amount of tryptophane present was measured by the growth response of Lactobacillus arabinosus 17-5 (Difco, 1953; Greene and Black, 1944).

Standard curves, as described in Part I, were constructed for each assay. A dl-standard was used for isoleucine (Difco, 1953), thus the values obtained from the assay required correction since the test organism responds only to the l-form (Schweigert et al. 1949).

The l-tryptophane standards were corrected with sodium hydroxide and heat, because the dl-isomers racemized during hydrolysis. L-amino acid standards were used to determine the amount of each of the eight remaining amino acids assayed. However, the lysine standard was made using the monohydrochloride form, and values were corrected to the pure l-isomer. Assay media for the amino acids were obtained from Difco Laboratories, Inc.

The frozen hydrolyzates were thawed as needed prior to assay and diluted to desired concentrations with distilled water. The test

samples were assayed in duplicate at four different concentration levels: 0.5, 1.0, 2.0, and 3.0 ml. Assay procedures including autoclaving, inoculation, incubation, and acidimetric titrations were similar to those previously described in Part I. The concentration of each amino acid in the samples was determined by reference to the standard curves shown in Figures 1-10. Only the segment of the standard curves used for determining the amino acid concentration in the actual assays were reproduced. Thus, a number of the standard curves appear as straight lines, while in reality they were hyperbolic curves.

Phenylalanine Recovery

Two one-gram samples of freeze-dried dark chicken meat and two one-gram samples of white chicken meat were hydrolyzed by refluxing with 6N hydrochloric acid for 16 hours in order to gain additional information concerning the low values obtained for phenylalanine in the microbiological assays of chicken meat.

Two of the hydrolyzates, one each of the dark and the white chicken meat, were filtered through a fritted glass filter in a glass Buchner funnel in order to remove humin (Gordon and Basch, 1964). The residues were washed with hot 1N hydrochloric acid.

The control samples, consisting of two hydrolyzates (one of dark chicken meat and one of white chicken meat) were clarified by the addition of activated charcoal as were the samples in Part I. The residues were washed with distilled water.

The recovery of standard phenylalanine was studied by adding 50 mg of L-phenylalanine to one gram samples of freeze-dried white chicken meat prior to hydrolysis. The recovery samples were hydrolyzed

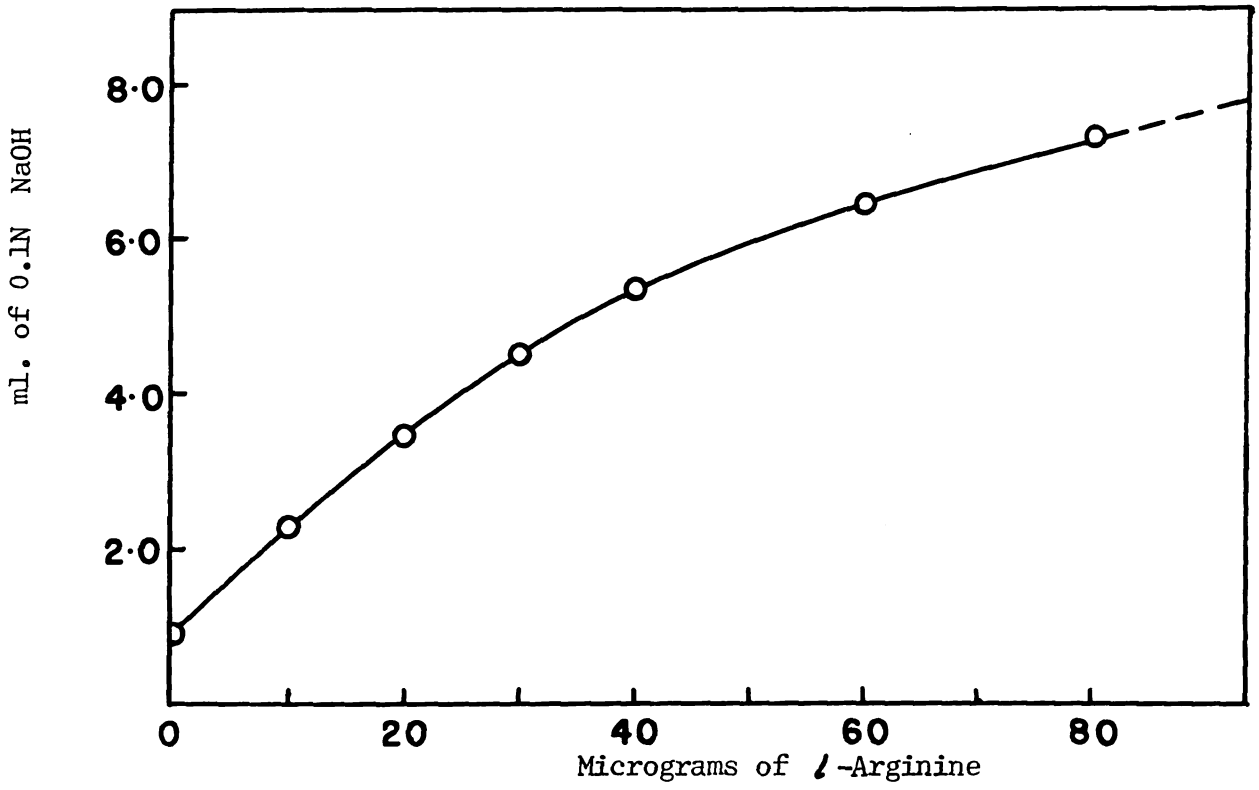

Fig. 1. Standard titration curve for l-Arginine.

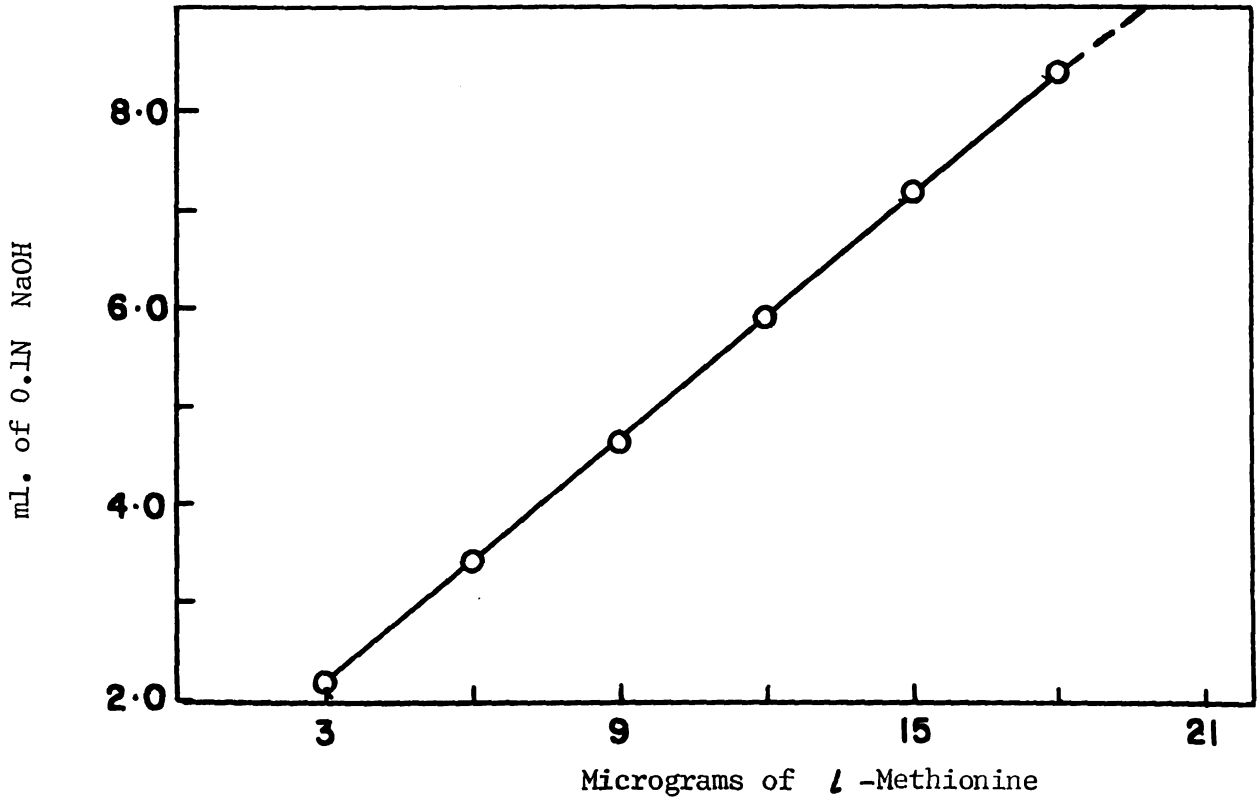

Fig. 2. Standard titration curve for l-Methionine.

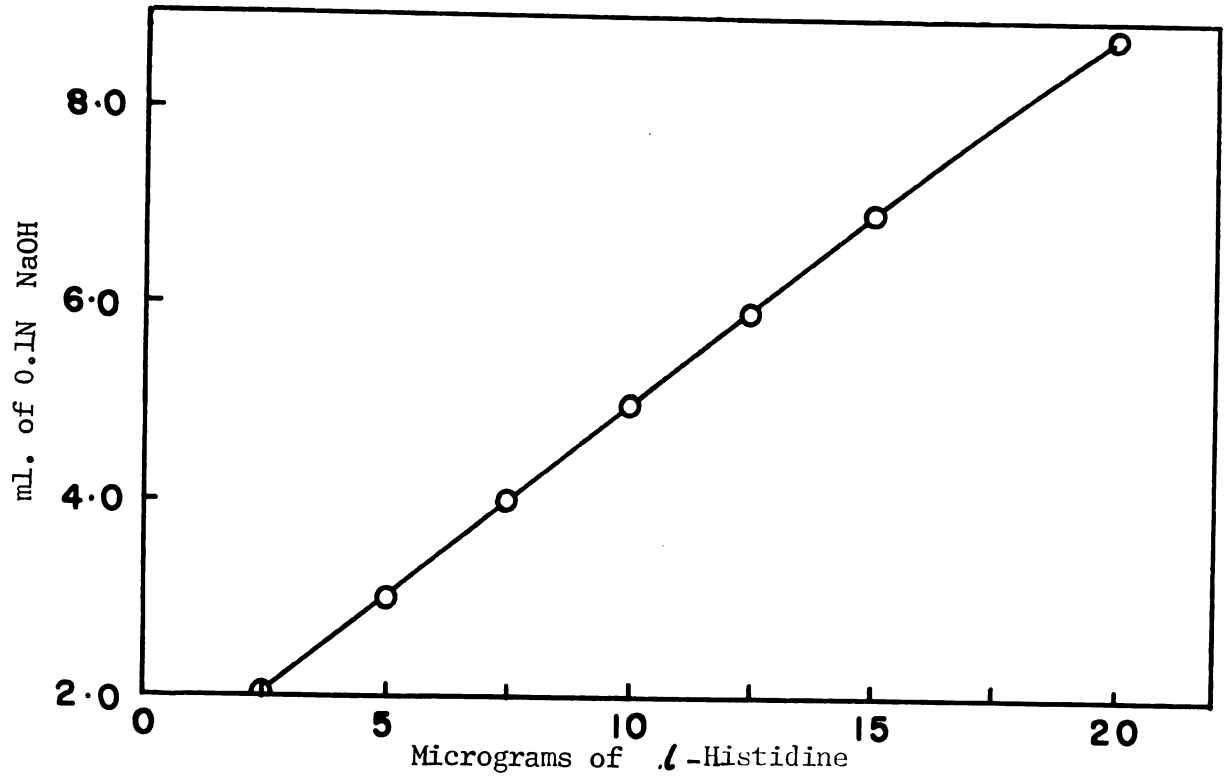

Fig. 3. Standard titration curve for l-Histidine.

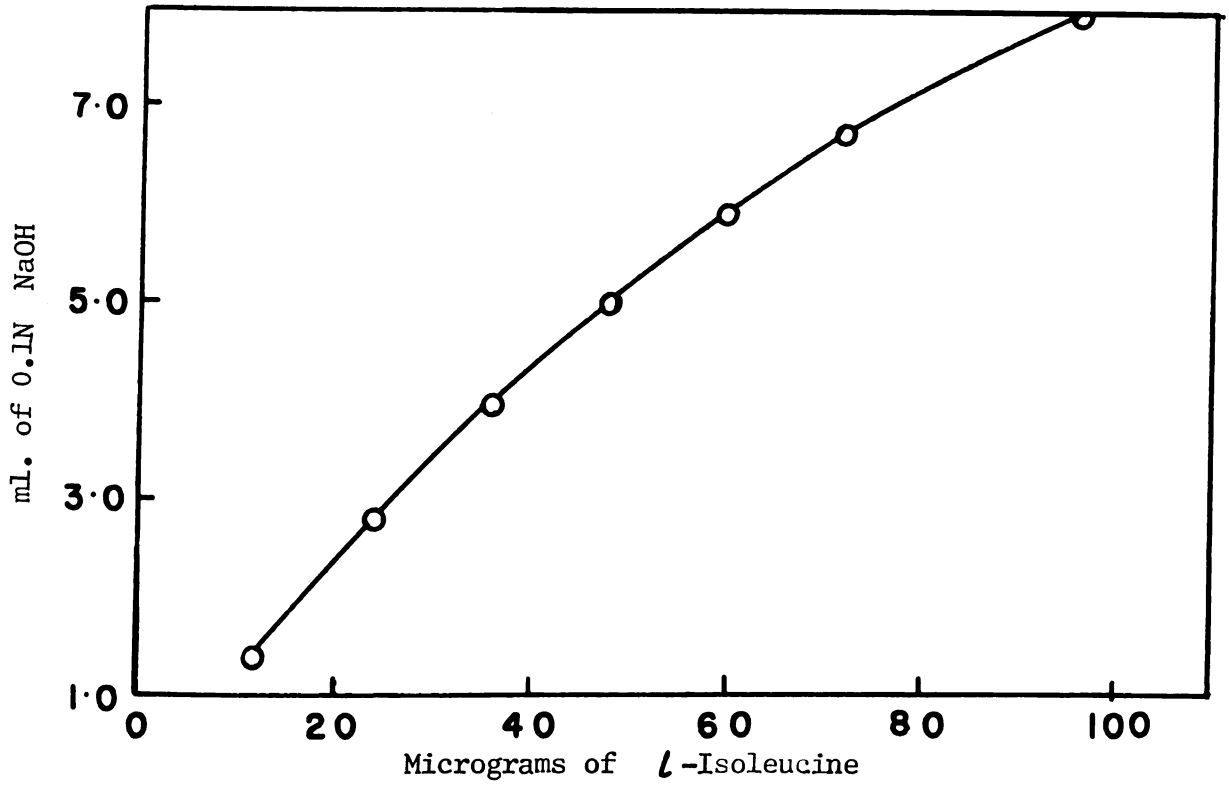

Fig. 4. Standard titration curve for l-Isoleucine.

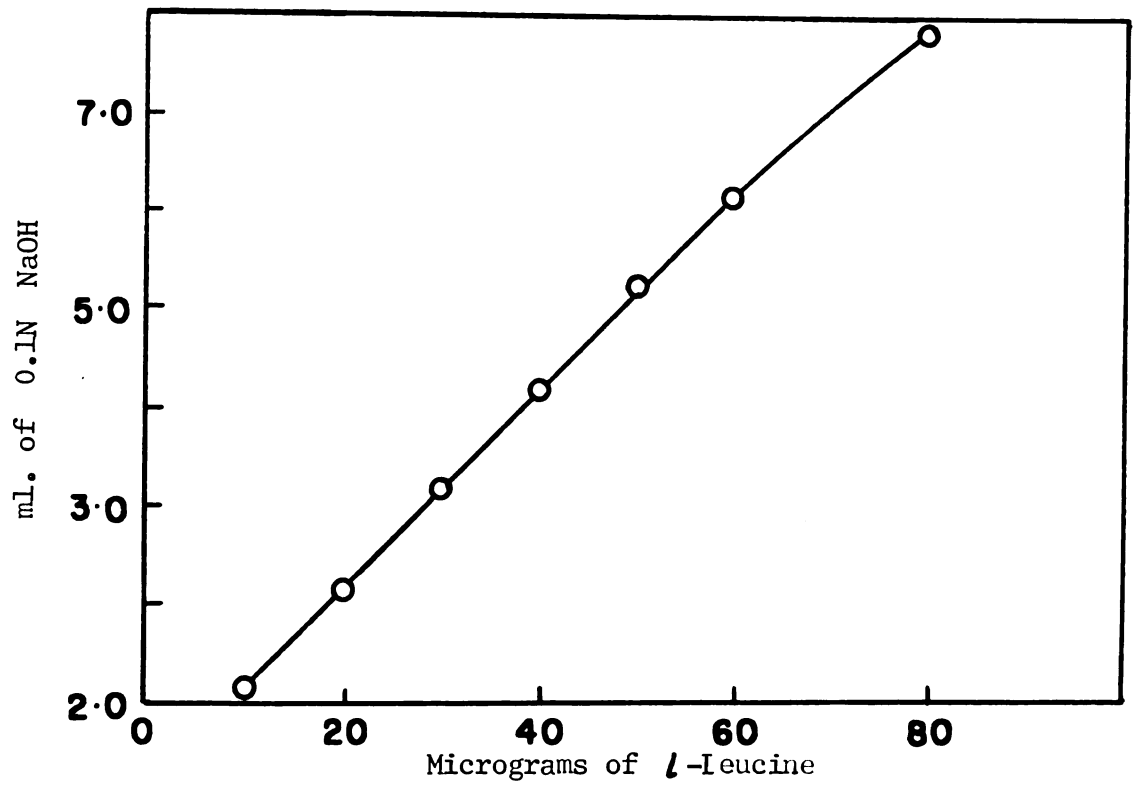

Fig. 5. Standard titration curve for *l*-Leucine.

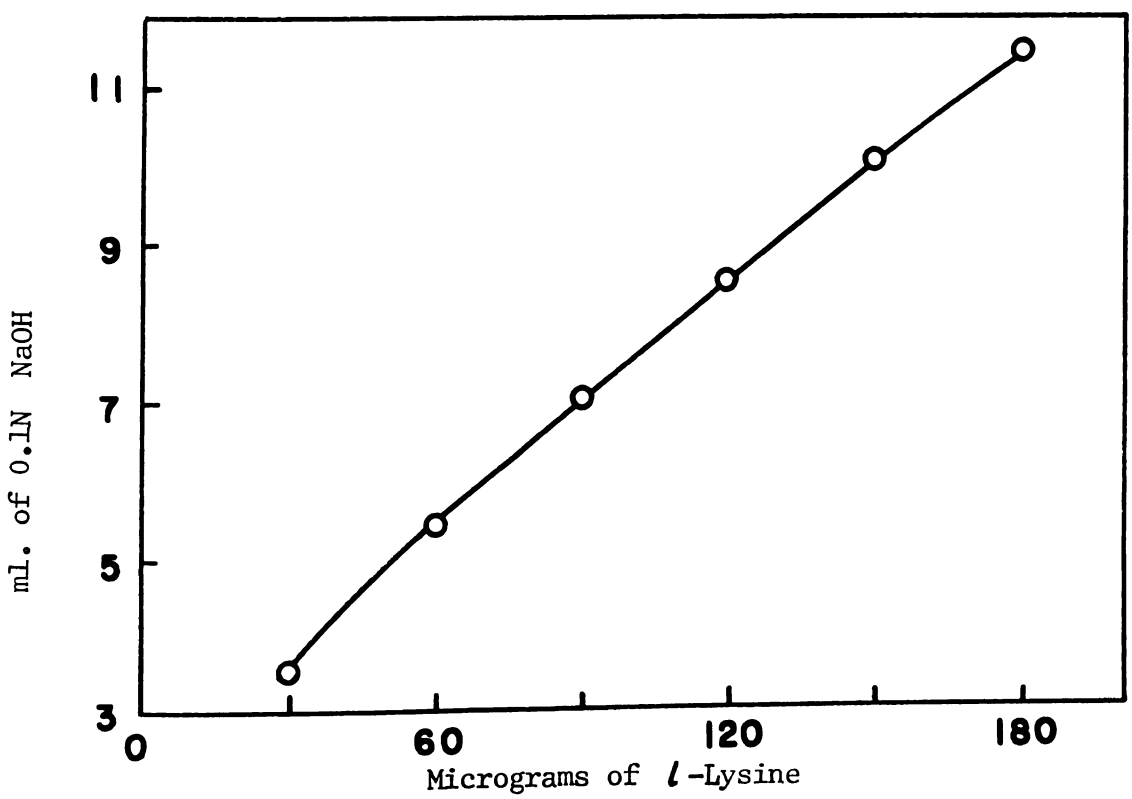

Fig. 6. Standard titration curve for *l*-Lysine.

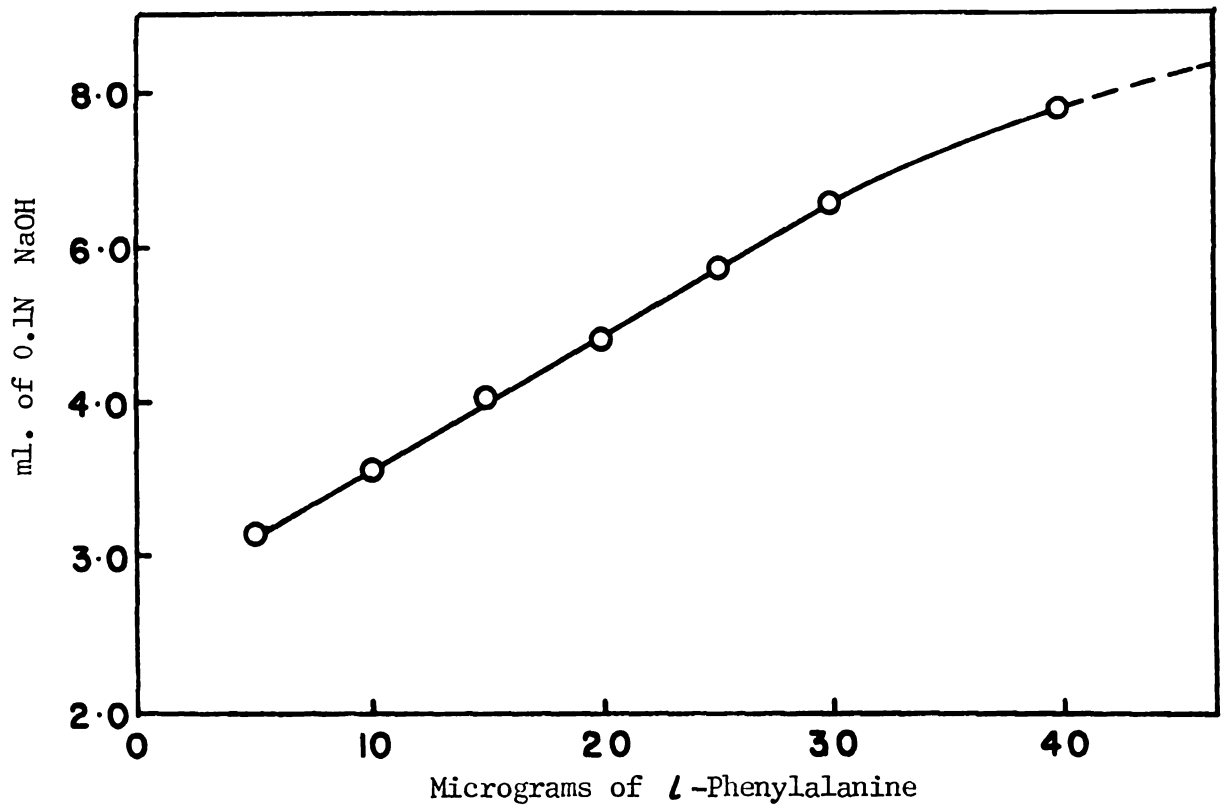

Fig. 7. Standard titration curve for l-Phenylalanine.

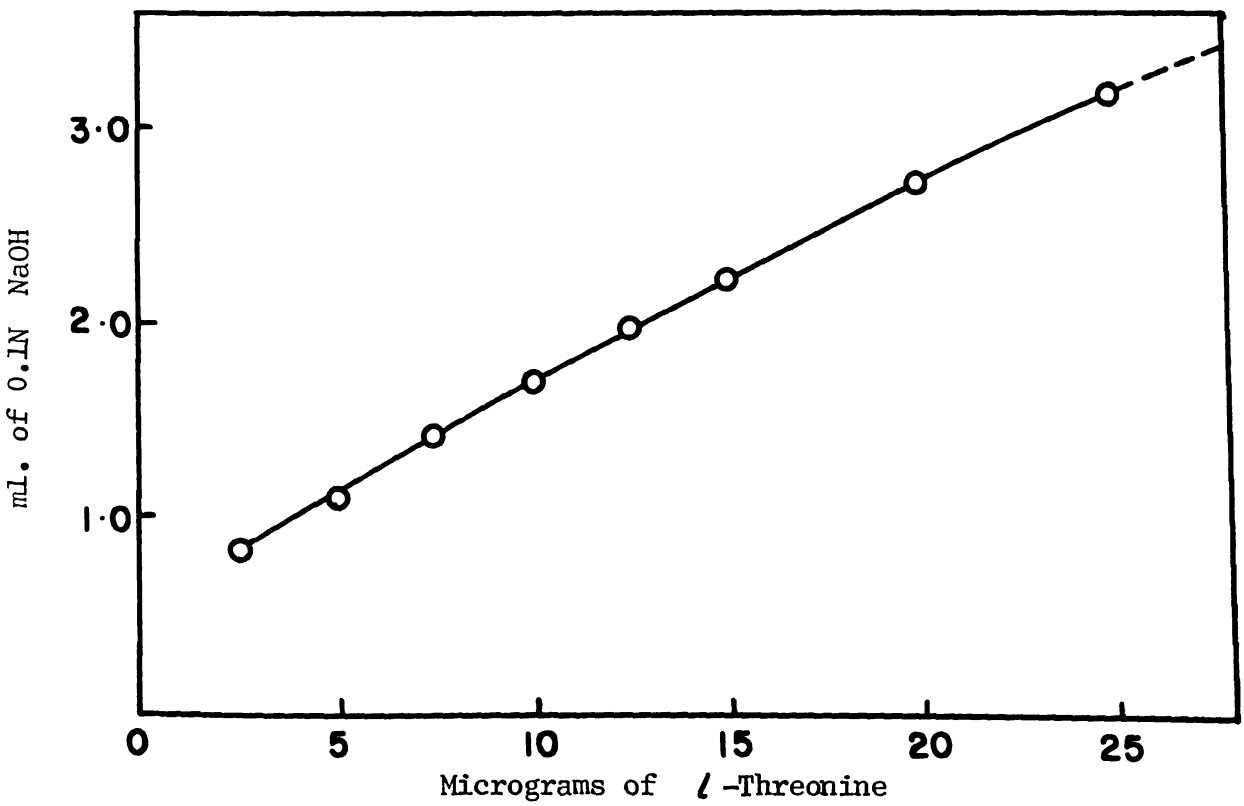

Fig. 8. Standard titration curve for l-Threonine.

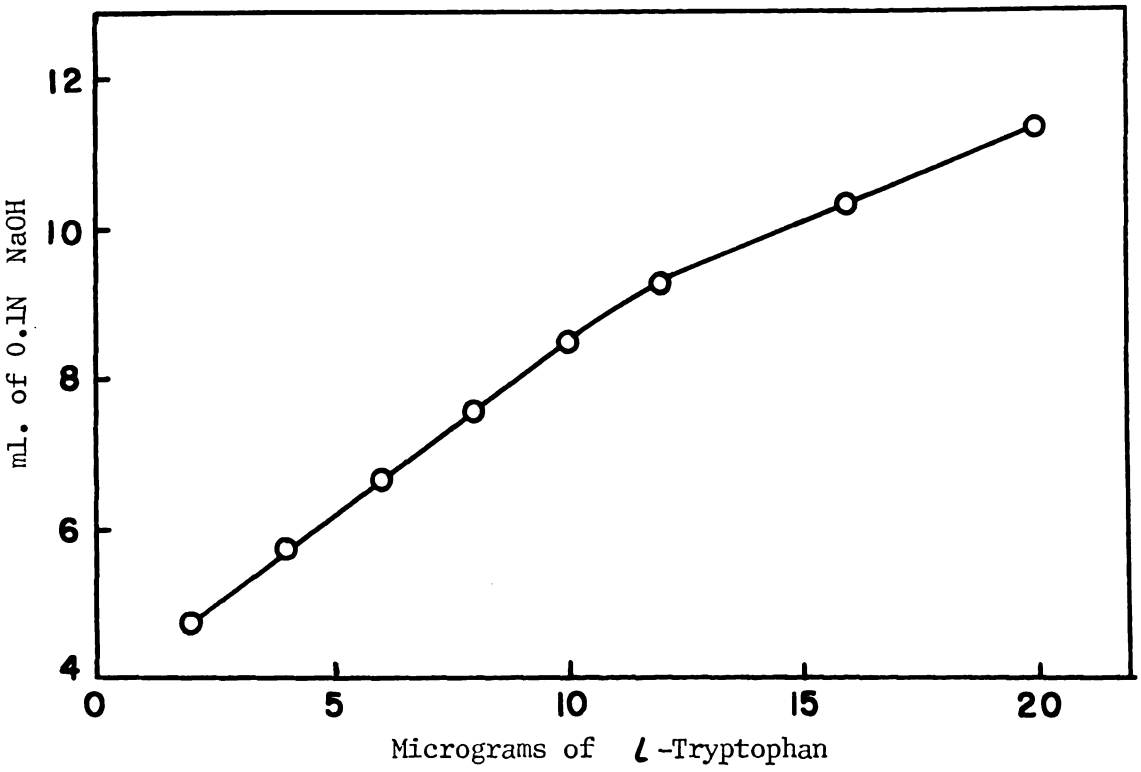

Fig. 9. Standard titration curve for l-Tryptophan.

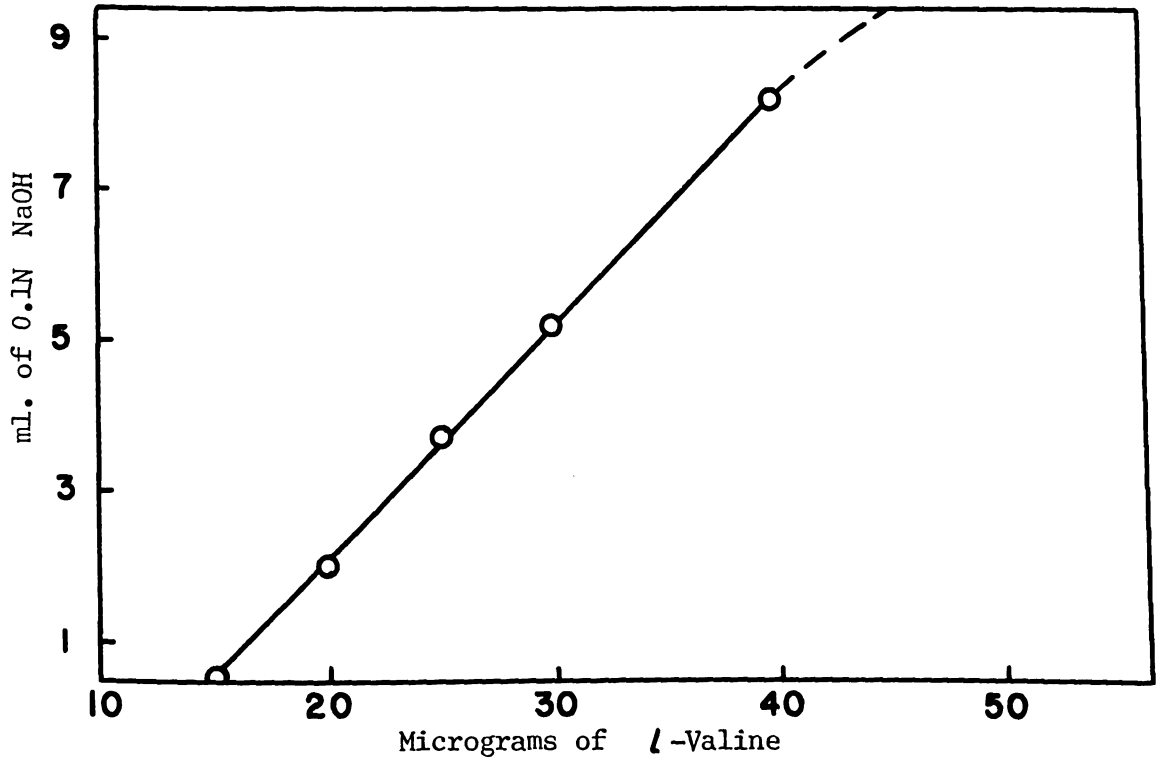

Fig. 10. Standard titration curve for l-Valine.

by refluxing with 6N hydrochloric acid for 16 hours. Hydrolyzates were clarified by the addition of activated charcoal and filtered; the residues were washed with distilled water.

All of the hydrolyzates were combined with their respective washings and neutralized with standard sodium hydroxide solution. The hydrolyzates were frozen in polyethylene bottles and held for analysis at 0°F. Phenylananine concentrations were determined by microbiological assay using L. mesenteroides as the test organism.

Part III

Amino Acid Analyzer

A Beckman/Spinco Model 120 Amino Acid Analyzer was used for the chromatographic determination of amino acids in chicken drip hydrolyzates. Samples were prepared according to the methods of Spackman (1960). The frozen drip hydrolyzates (dark and white meat, 30 and 90 day storage) were thawed at 35-40°F. Aliquots varying in size from 25-35 ml were transferred to 100 ml round bottom flasks. The samples were evaporated to dryness in a rotary evaporator under vacuum in approximately 20-30 minutes. The dried film was washed with distilled water, and the samples were dried again. A quantity of distilled water was added to dissolve the dried material; the final solutions contained 3.5 ± 0.5 mg of protein per ml. These samples were transferred to 4 ounce polyethylene bottles, tagged for identification, frozen at -20°F and held at 0°F until analyzed.

For analysis, the samples were thawed and brought to room temperature. One ml of the protein solution was diluted to 5 ml with buffer (pH 2.2). Two 2-ml aliquots were used for analysis on the analyzer, one for the determination of neutral and acidic amino acids

and one for the basic acids.

Total nitrogen content of the protein solutions prepared for analysis on the analyzer were checked by the micro-Kjeldahl method.

RESULTS AND DISCUSSION

Part I

The effects of time and method of hydrolysis on the liberation of arginine, leucine and methionine from dark chicken meat are presented in Figures 11-13. The maximum liberation of all three amino acids, as determined by microbiological assay, were obtained by refluxing with 6N HCl. Under the conditions of this experiment, autoclaving with 2N HCl resulted in higher values than those values obtained by hydrolyzing in sealed tubes with 3N HCl.

Maximum liberation of arginine and leucine was obtained in 16 hours. With methionine, an 8 hour hydrolysis resulted in a maximum release of 2.31 grams of amino acid per gram of protein; a 16 hour hydrolysis liberated slightly less methionine (2.25 grams per 100 grams of protein).

A 16 hour refluxing time with 6N HCl was selected for the hydrolysis of chicken meat. These conditions resulted in maximum release of arginine and leucine and near maximum liberation of methionine under the conditions of this study.

All three methods of hydrolysis (refluxing, sealed tube, autoclaving) for the three amino acids, with the exception of the arginine-sealed tube hydrolysis, resulted in decreased liberation of the amino acids after 24 hours of hydrolysis as compared to the values at 16 hours.

Drip from dark chicken meat was hydrolyzed by refluxing with 6N HCl in order to eliminate variability due to method of hydrolysis. Hydrolysis times of 2, 4, 8 and 16 hours were used to determine the time required for maximum liberation of arginine, leucine and methionine. The results presented in Figure 14 indicate that maximum amino acid

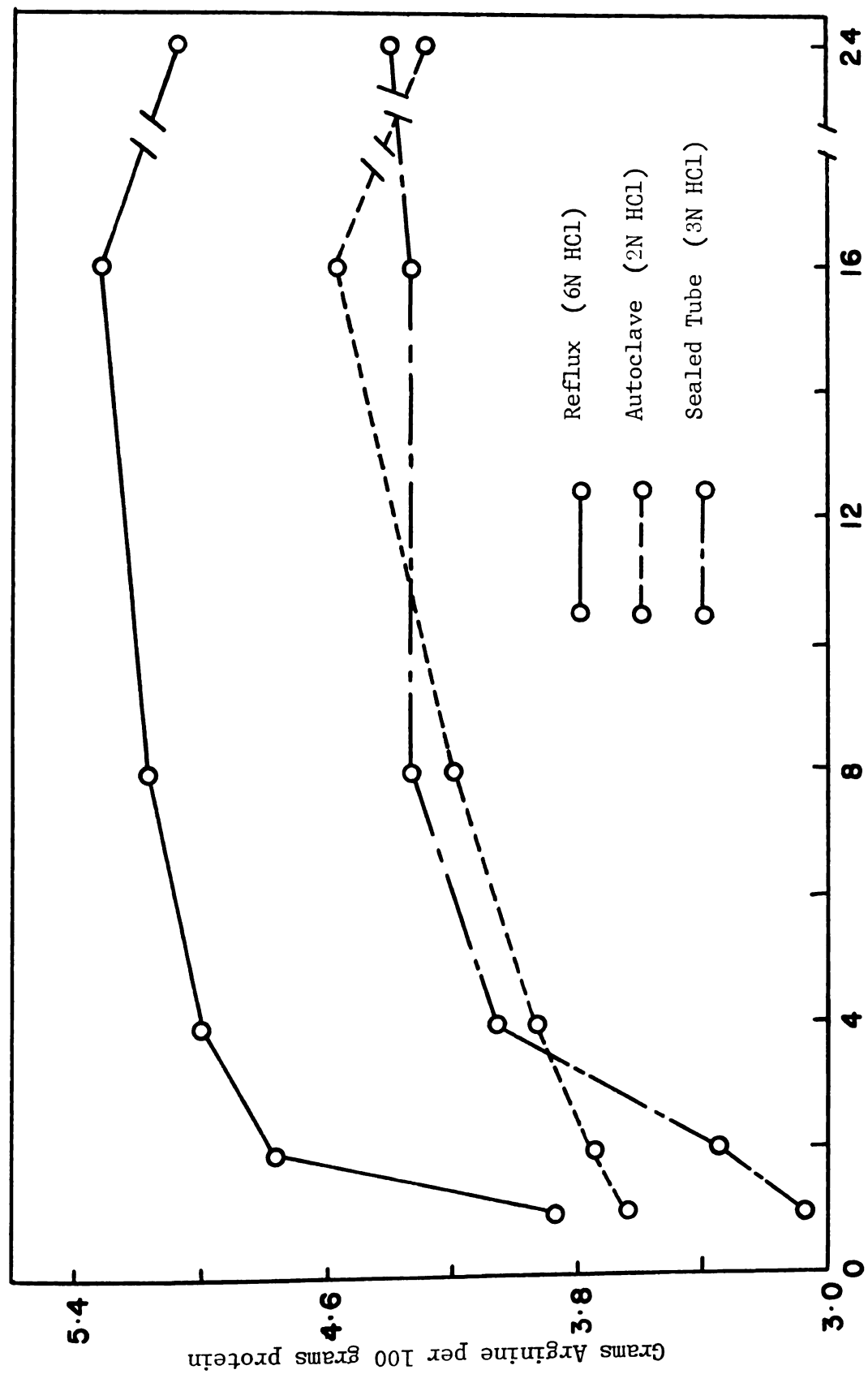

Fig. 11. Effect of time of hydrolysis on Arginine values obtained from chicken . . .(dark meat).

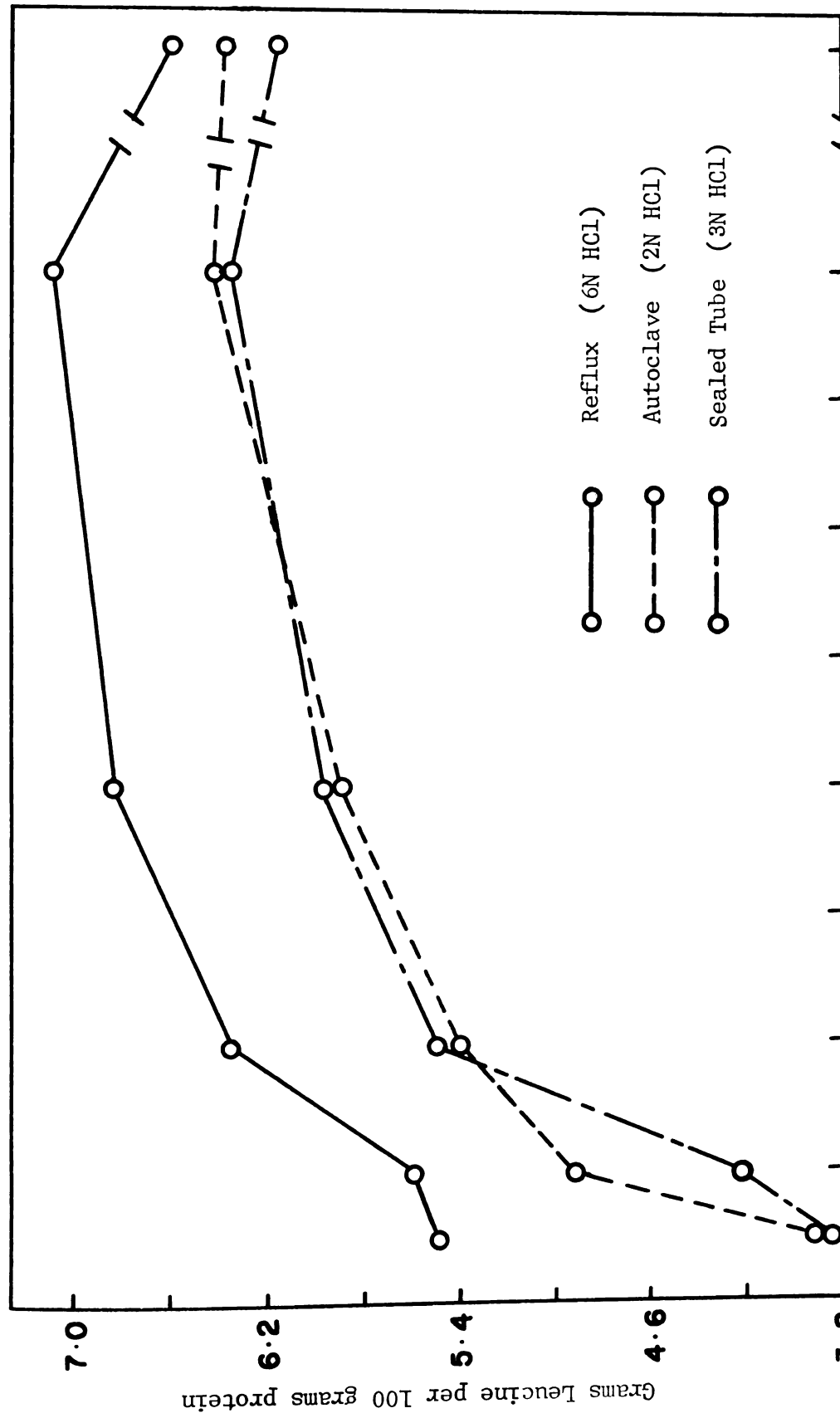

Fig. 12. Effect of time of hydrolysis on Leucine values obtained from chicken. . . (dark meat).

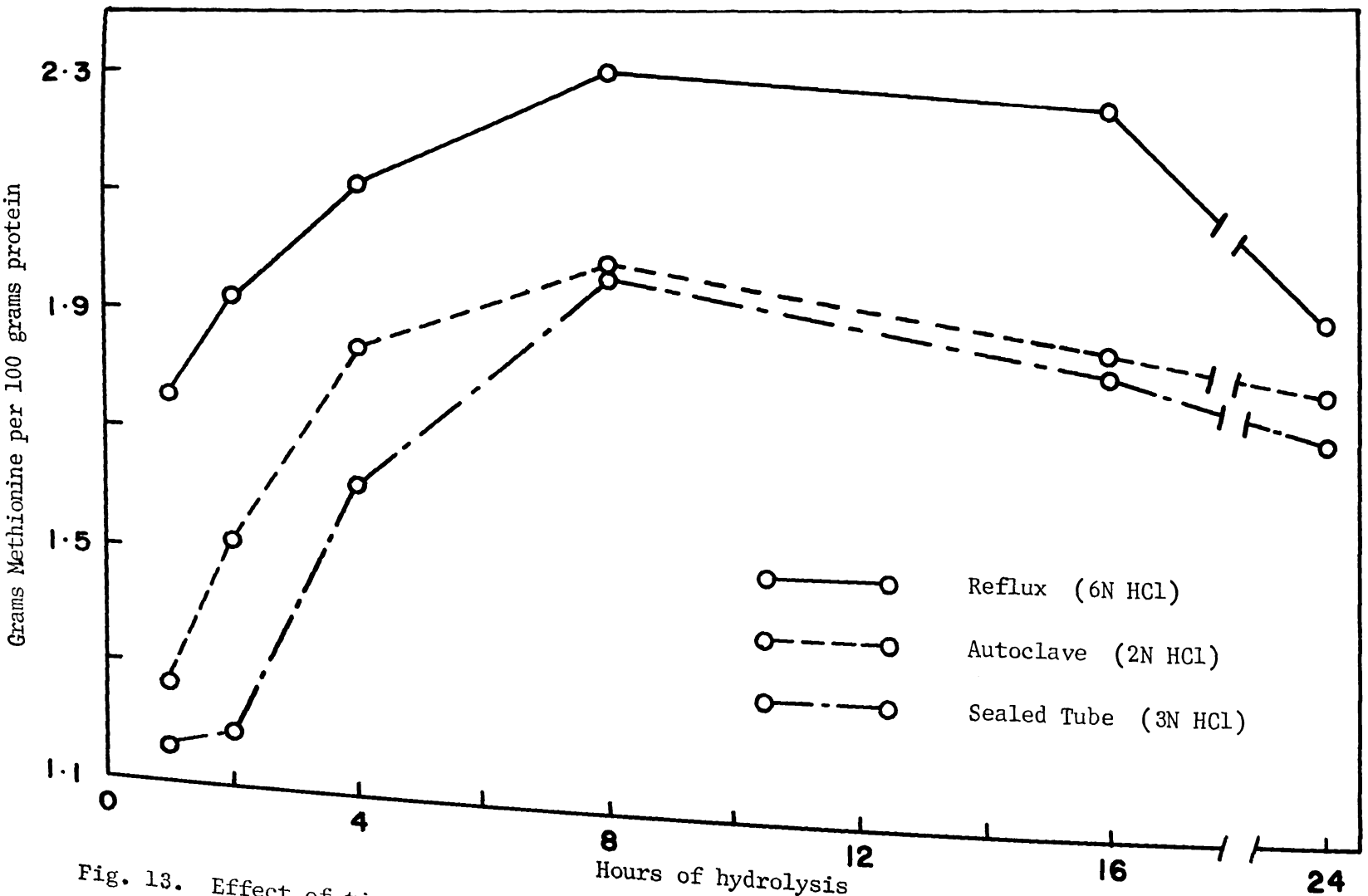

Fig. 13. Effect of time of hydrolysis on Methionine values obtained from chicken . . .(dark meat)

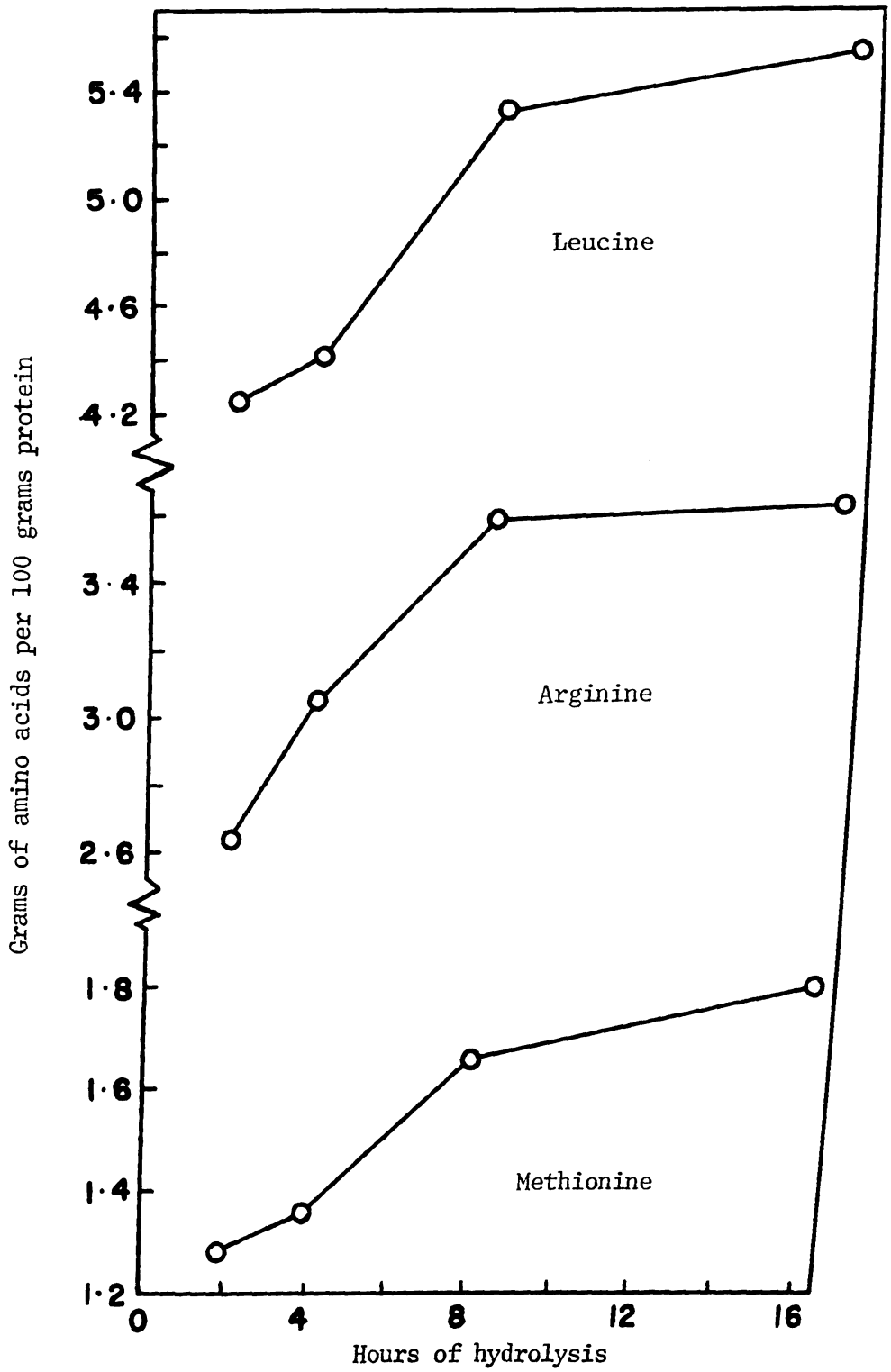

Fig. 14. Effect of time of hydrolysis on specific amino acid values obtained from chicken drip.

values are obtained with a 16 hour hydrolysis for all three amino acids as determined by microbiological assay.

The growth response on L. mesenteroides produced by a NaCl-standard amino acid solution was compared over a 10-fold range with a standard amino acid solution of the same concentration. From the results shown in Table 1 one may conclude that no inhibition was detected at the concentration levels used for leucine and methionine. With arginine a slight decrease in growth response, as measured by titratable acidity is indicated at a concentration of 60 micrograms. This inhibition becomes more pronounced at 80 and 100 microgram concentrations. However, this was not considered detrimental to the assay as most readings were made on the first part of the standard curves (concentrations of 10-60 micrograms).

The salt concentration ranges used for leucine and methionine were 0.14-1.40 percent and 0.35-3.50 percent, respectively. However, a more concentrated salt range of 0.70-7.00 percent was used for arginine; this may have contributed to the slight inhibition of growth at the higher standard concentrations.

Part II

Water, fat, total nitrogen and protein nitrogen determinations were made on dark and white chicken meat at various stages of sample preparation for hydrolysis. The results are reported in Table 2.

Water content of the meat samples was determined after frozen storage periods of 30 and 90 days. The white meat (73.2 and 72.1 percent) contained slightly less water than the dark meat (74.3 and 74.4 percent) after both 30 and 90 days of frozen storage. These results were essentially in agreement with those of Millares and Fellers (1948) and

Table 1. Standard titration values[1/] for specific amino acids with and without the addition of NaCl to the standard amino acid solution

Amino acid	Concentration µg/tube	Ml. of 0.1N NaOH	
		Standard	Standard + NaCl
Methionine	0.0	0.75	0.70
	3.0	1.85	1.85
	6.0	2.85	2.95
	9.0	3.85	4.00
	12.0	4.85	5.00
	15.0	5.85	6.00
	18.0	6.75	6.85
	24.0	8.20	8.40
	30.0	9.55	9.70
Leucine	0.0	0.75	0.75
	10.0	2.20	2.20
	20.0	3.45	3.50
	30.0	4.65	4.65
	40.0	5.85	5.75
	50.0	6.75	6.85
	60.0	7.85	7.70
	80.0	9.45	9.50
	100.0	11.05	11.05
Arginine	0.0	1.00	1.00
	10.0	2.25	2.25
	20.0	3.45	3.50
	30.0	4.55	4.55
	40.0	5.40	5.40
	50.0	6.05	6.00
	60.0	6.60	6.50
	80.0	7.30	6.95
	100.0	7.80	7.10

1/ By microbiological assay.

Table 2. Composition of dark and white chicken meat after 30 and 90 days of frozen storage

Sample	Frozen storage (days)	Nitrogen 1/ (%)	Protein 1/ (%)	Fat 2/ (%)	Water 3/ (%)
Dark	30	13.7	85.5	18.3	74.8
	90	13.8	86.1	20.3	74.4
White	30	14.1	88.3	3.9	73.2
	90	14.4	90.2	5.7	72.1

1/ Average of two samples each containing three replicates expressed as percent of freeze-dried, ether-extracted meat.

2/ Average of two samples each containing four replicates expressed as percent of freeze-dried meat.

3/ Average of two samples each containing three replicates expressed as percent of thawed meat.

Minor (1964).

Values obtained for water content of both dark and white meat indicated a slight decrease during storage between 30 and 90 days. However, differences were not measured statistically. The water content of dark meat decreased from 73.2 to 72.1 percent during storage. Hepburn (1950) similarly indicated that increased periods of frozen storage would result in decreased water content and that the decrease in white meat was usually greater than in dark meat.

Fat content was determined for dark and white chicken meat which had been freeze-dried after 30 and 90 days of frozen storage. The dark meat contained 18.3 and 20.3 percent fat which was slightly more than that in the white meat (3.9 and 5.7 percent). The relative amounts of fat from dark and white meat are in general agreement with results reported by Millares and Fellers (1948) and Hepburn (1950).

The nitrogen and protein nitrogen determinations were carried out on freeze-dried dark and white meat samples following fat removal by ether extraction. The samples of dark meat stored 30 and 90 days contained 85.5 and 86.1 percent protein respectively; the white meat samples contained 88.3 and 90.2 percent protein. These values are in agreement with results of Millares and Fellers (1948), Minor (1964) and Hepburn (1950).

The percentages of drip obtained from dark and white chicken meat after frozen storage periods of 30 and 90 days are presented in Table 3. The percentage drip from dark meat increased from 4.8 to 7.9 percent as the frozen storage period was increased from 30 to 90 days. Drip from the white meat increased from 5.4 to 9.6 percent with increased periods of frozen storage. Larger quantities of drip

Table 3. Percent drip obtained from dark and white chicken meat after frozen storage periods of 30 and 90 days

Meat sample		Frozen storage (days)	Wt. of meat (g)	Vol. of drip (ml)	Drip (%)
Dark	A	30	394.0	21.2	5.4
	B	30	406.0	16.6	4.1
	Mean				4.8
Dark	A	90	345.0	23.3	6.8
	B	90	363.0	32.6	9.0
	Mean				7.9
White	A	30	311.0	18.3	5.9
	B	30	412.0	20.0	4.9
	Mean				5.4
White	A	90	310.0	28.0	9.0
	B	90	368.0	37.2	10.1
	Mean				9.6

were obtained from the white meat than from the dark meat after frozen storage periods of both 30 and 90 days.

The protein nitrogen content of drip obtained from the dark and white meat held for storage periods of 30 and 90 days are reported in Table 4. Under the conditions of this study, drip from the white meat contained greater quantities of protein than drip from dark meat. The drip from white meat held for 90 days contained 10.92 percent protein nitrogen; the drip from white meat in storage for 30 days contained 9.17 percent. Similarly, the protein nitrogen content of drip from dark meat stored for 90 days (6.26 percent) was slightly larger than the protein content of drip from dark meat held for 30 days, (5.16 percent).

Although the increase in protein nitrogen which occurred in the drip from dark and white meat is of limited proportion, it does indicate the possibility of denaturation and proteolysis in the chicken meat during frozen storage. Khan et al. (1963) reported that denaturation and proteolysis occurred in chicken meat muscle proteins in frozen storage at -18°, -10° and -4°C. Swanson and Sloan (1953) concluded that proteolysis in poultry was indicated during frozen storage by an increase in soluble nitrogen and non-protein nitrogen of both dark and white meat.

The pH values for dark and white chicken meat after slaughter, and following 90 days of frozen storage are presented in Table 5. The dark and white meats of the chicken had pH values of 6.25 and 5.90, respectively, approximately two hours after slaughter. Following 90 days of frozen storage, the pH values of dark meat from the intact carcass decreased to approximately 6.15 while the pH of white meat decreased to

Table 4. Average[1/] protein and nitrogen content of drip obtained from dark and white chicken meat held for storage periods of 30 and 90 days

Sample		Frozen storage (days)	Nitrogen (%)	Protein (%)
Dark	A	30	0.96	5.99
	B	30	0.69	4.33
	Mean		0.83	5.16
Dark	A	90	1.12	6.97
	B	90	0.89	5.55
	Mean		1.01	6.26
White	A	30	1.30	8.13
	B	30	1.63	10.20
	Mean		1.47	9.17
White	A	90	1.62	10.01
	B	90	1.89	11.83
	Mean		1.76	10.92

1/ Average of three replicates.

Table 5. pH values[1/] for dark and white chicken meat

Treatment	Intact bird		Separated	
	dark	white	dark	white
Two hours post mortem	6.25	5.90	- -	- -
90 day frozen storage	6.15	5.85	6.10	5.75

1/ Average of four samples each containing two replicates.

5.85. Separated dark and white meat frozen in individual packages and stored frozen for 90 days had pH values of 6.1 and 5.75, respectively.

Of specific interest is the comparative pH values of dark and white meats after frozen storage. In both instances (intact carcass and separated packaged meat) the dark meat had higher pH values than the white meat. In addition a comparison of percentage drip from dark and white meat after 90 days frozen storage (Table 3) indicates that the white meat exuded a larger quantity of drip (9.6 percent) than the dark meat (7.6 percent). More drip was obtained from the meat with the lower pH value.

A direct relationship between pH values and drip has been amply confirmed by a number of workers. Empey (1933) clearly demonstrated that a high ultimate pH was associated with diminished drip loss from beef muscles. Sair and Cook (1938 b) reported that, with precooled meats, the maximum amount of drip was obtained at about pH 5.2; as the pH increased the amount of drip decreased to zero at about pH 6.4. Kuprianoff (1952) indicated that meat with a pH near its isoelectric point (5.0 to 5.5) has a greater drip loss after freezing and thawing than meat at a higher pH (6.3 to 6.4).

The essential amino acids in dark and white chicken meat and drip after 30 and 90 days of frozen storage as determined by microbiological assay are presented in Tables 6 to 9. Tables 6 and 8 report the essential amino acid composition of dark and white chicken meat after 30 days of frozen storage. The values obtained in this investigation agree with those of Millares and Fellers (1948) who reported the amino acid content of frozen raw dark and white chicken meat; and Beach et al. (1943) who included analysis of both dark and

Table 6. Essential amino acids in dark chicken meat and drip after 30 days frozen storage and the percentage of the total contained in the drip

Amino acid [1/]	Meat g/100 g protein	Drip g/100 g protein	Percentage of total contained in drip
Arginine	6.08	2.70	2.05
Histidine	2.24	1.36	2.89
Isoleucine	5.30	2.77	2.45
Leucine	8.58	5.59	3.07
Lysine	8.88	5.11	2.74
Methionine	2.48	1.23	2.32
Phenylalanine	0.81	0.14	0.79
Threonine	4.13	2.33	2.66
Tryptophane	1.18	1.45	3.82
Valine	5.04	4.97	4.63
Average [2/]			2.95

1/ Microbiological assay.

2/ Average does not include phenylalanine.

Table 7. Essential amino acids in dark chicken meat and drip after 90 days frozen storage and the percentage of the total contained in the drip

Amino acid [1/]	Meat g/100 g protein	Drip g/100 g protein	Percentage of total contained in drip
Arginine	5.32	2.94	4.36
Histidine	1.66	1.99	9.43
Isoleucine	4.50	3.56	6.22
Leucine	7.06	6.06	6.69
Lysine	7.97	6.14	6.12
Methionine	1.93	1.41	5.72
Phenylalanine	0.33	0.15	3.57
Threonine	2.61	2.90	8.86
Tryptophane	1.07	1.43	10.84
Valine	4.71	5.64	9.47
Average [2/]			7.58

1/ Microbiological assay.

2/ Average does not include phenylalanine.

Table 8. Essential amino acids in white chicken meat and drip after 30 days frozen storage and the percentage of the total contained in the drip

Amino acid 1/	Meat g/100 g protein	Drip g/100 g protein	Percentage of total contained in drip
Arginine	5.68	3.30	3.12
Histidine	3.13	3.23	5.51
Isoleucine	5.50	4.26	4.16
Leucine	8.83	6.27	3.82
Lysine	8.99	6.25	3.72
Methionine	2.41	1.49	3.31
Phenylalanine	0.57	0.19	1.88
Threonine	4.06	3.18	4.19
Tryptophane	1.34	1.02	4.09
Valine	5.89	5.56	5.04
Average 2/			4.11

1/ Microbiological assay.

2/ Average does not include phenylalanine.

Table 9. Essential amino acids in white chicken meat and drip after 90 days frozen storage and the percentage of the total contained in the drip

Amino acid [1/]	Meat g/100 g protein	Drip g/100 g protein	Percentage of total contained in drip
Arginine	4.31	3.49	7.73
Histidine	2.58	3.54	13.11
Isoleucine	4.76	4.84	9.67
Leucine	7.42	6.36	8.18
Lysine	7.91	7.10	8.57
Methionine	1.90	1.73	8.71
Phenylalanine	0.22	0.24	10.11
Threonine	3.12	3.22	9.86
Tryptophane	1.28	1.27	9.48
Valine	4.91	6.47	12.60
Average [2/]			9.77

1/ Microbiological assay.

2/ Average does not include phenylalanine.

white chicken meat cooked prior to analysis.

Values obtained for leucine, valine, lysine and threonine in this study were somewhat higher than values reported by Millares and Fellers (1948), while values for histidine and methionine were somewhat lower. Close agreement was obtained for the arginine, isoleucine and tryptophane values.

The values reported by Beach et al. (1943) were higher than most of the values obtained in this study. Tryptophane values were in close agreement while the histidine values obtained in this investigation were somewhat higher. Variations could readily be attributed to differences in the samples used (age or strain of the chicken) or the methodology employed.

The values obtained for phenylalanine throughout this study have been reported but are unduly low. Factors pertaining to these low results are discussed in the following sections and further discussion of phenylalanine is omitted until that time.

The concentrations of essential amino acids in dark chicken meat drip after frozen storage of 30 and 90 days and the percentages of the total amino acids in meat, which were contained in the drip, are presented in Tables 6 and 7. The percentage loss in drip from meat held for 30 days ranged from 2.05 percent for arginine to 4.63 percent for valine. Losses for seven of the amino acids, as measured in drip, ranged from 2.05 to 3.07 percent. The losses of tryptophane (3.82 percent) and valine (4.63 percent) were somewhat larger. The average loss for nine amino acids, excluding phenylalanine, was 2.95 percent.

The percentages of total amino acids contained in the drip from dark meat after 90 days of storage (Table 7) ranged from 4.36

percent for arginine to 10.84 percent for tryptophane, with an average for nine amino acids of 7.58 percent. The concentrations of seven of these amino acids ranged from 5.72 to 9.47 percent.

In Tables 8 and 9 are presented the amounts of essential amino acids in the drip from white meat held in frozen storage for periods of 30 and 90 days. The quantities of nine amino acids from 30 day frozen samples (Table 8) ranged from 3.12 percent for arginine to 5.51 percent for histidine with an average of 4.11 percent.

The values for amino acids from white meat held for 90 days frozen storage (Table 9) ranged from 7.73 percent for arginine to 13.11 percent for histidine. The concentrations of seven of the amino acids were between 7.73 and 9.86 percent, and the average content of nine amino acids was 9.77 percent.

The amount of amino acids in the drip from white and dark meats after 30 and 90 days frozen storage increased with extended periods of frozen storage. This is verified by the increase in average amino acid content in drip from both the white meat samples (2.95 to 7.58 percent) and the dark meat samples (4.11 to 9.77 percent). This increase in amino acids in drip was suggested by the values for protein nitrogen for dark and white samples at 30 and 90 days frozen storage (Table 4). The protein nitrogen content of drip from dark meat increased from 5.16 to 6.26 percent during storage while the protein content of the drip from white meat increased from 9.17 to 10.92 percent.

The higher values for amino acids from white meat (30 and 90 days frozen storage) as compared to dark meat is also reflected in the protein contents of the original drip samples. The average amino

acid content for drip from white and dark meat at 30 days of storage was 4.11 percent and 2.95 percent respectively, while the values after 90 days of storage were 9.77 and 7.58 percent. Protein contents of drip from white and dark meat after 30 days of storage were 9.17 percent and 5.16 percent; the values at 90 days were 10.92 percent and 9.17 percent.

Table 10 presents the concentrations of phenylalanine contained in the hydrolyzates of dark and white chicken meat. The hydrolyzates had been filtered through a glass fiber filter to remove humin prior to analysis. Also included in the table are recovery values for phenylalanine standards which were clarified by passage through activated charcoal following hydrolysis. Both determinations were carried out by microbiological assay. The concentrations of phenylalanine detected in the glass filtered samples were 3.45 g per 100 g of protein in the dark meat and 3.64 g per 100 g of protein in the white meat. The value for white meat is in good agreement with that reported by Millares and Fellers (1948) while the value for dark meat is somewhat lower.

The quantity of phenylalanine standard recovered was 61.0 g per 100 g of protein. This limited investigation indicates that decolorization with activated charcoal removed 39.5 percent of the phenylalanine. Featherstone *et al.* (1964) reported a 30.6 percent loss of phenylalanine as determined by an amino acid analyzer.

The low phenylalanine values obtained in this study can be partly attributed to the loss of phenylalanine on decolorization. The results obtained using the glass filtered hydrolyzate indicate that this method warrants consideration in phenylalanine determinations.

Table 10. Concentrations of phenylalanine in glass filtered[1/] hydrolyzate from white and dark chicken meat and loss of phenylalanine standard from clarified[2/] hydrolyzate

Treatment	µg/ml	g/100 g protein	Loss (%)
Filtered dark	11.50	3.45	- -
white	12.40	3.64	- -
Recovery standard[3/]	2.48	61.0	39.5

1/ Filtered through a glass fiber filter.

2/ Clarified by the addition of 1g of activated carbon per 100 ml of hydrolyzate.

3/ Average of two samples.

Part III

The concentration of essential amino acids in drip obtained from white and dark chicken meat held for frozen storage periods of 30 and 90 days as determined by an amino acid analyzer are presented in Tables 11 to 14.

Results for phenylalanine are not reported because of the loss of this amino acid in preparation of the hydrolyzates. Tryptophane is not resolved by the methods used in this part of the study. The concentrations of the essential amino acid in dark and white meat for both periods of storage (30 and 90 days) as determined by microbiological assay are included in these tables to facilitate the determination of percentage of total amino acids in meat which are obtained in the drip.

Eight of the essential amino acid values for drip from dark meat held in storage for 30 days are reported in Table 11. The concentrations of these amino acids range from 2.88 percent for arginine to 6.05 percent for histidine, with an average value of 3.80 percent.

The concentrations of eight essential amino acids in drip from dark meat held for 90 days of frozen storage (Table 12) ranged from 4.70 percent for arginine to 12.83 percent for histidine. However, six of these amino acids had values in the range of 5.80 to 9.88 percent, while the average value was 8.01 percent.

Table 13 contains the percentages of amino acids in drip from white meat after frozen storage of 30 days. The concentrations ranged from 3.63 percent (arginine) to 7.26 percent (histidine) with six of the amino acids within a range of 4.05 to 5.45 percent. The average

Table 11. Essential amino acids in dark chicken meat and drip after 30 days frozen storage and the percentage of the total contained in the drip

Amino acid	Meat[1/] g/100 g protein	Drip[2/] g/100 g protein	Percentage of total contained in drip
Arginine	6.08	3.72	2.80
Histidine	2.24	2.94	6.05
Isoleucine	5.30	3.06	2.72
Leucine	8.58	6.32	3.49
Lysine	8.79	7.50	4.03
Methionine	2.47	1.56	3.01
Threonine	4.13	2.98	3.41
Valine	5.04	5.20	4.86
Average			3.80

1/ Microbiological assay.

2/ Amino acid analyzer.

Table 12. Essential amino acids in dark chicken meat and drip after 90 days frozen storage and the percentage of the total contained in the drip

Amino acid	Meat 1/ g/100 g protein	Drip 2/ g/100 g protein	Percentage of total contained in drip
Arginine	5.32	3.17	4.70
Histidine	1.66	2.70	12.83
Isoleucine	4.50	3.31	5.80
Leucine	7.06	6.51	7.24
Lysine	7.97	7.86	7.81
Methionine	1.93	1.55	6.38
Threonine	2.61	3.22	9.88
Valine	4.71	5.63	9.43
Average			8.01

1/ Microbiological assay.

2/ Amino acid analyzer.

Table 13. Essential amino acids in white chicken meat and drip after 30 days frozen storage and the percentage of the total contained in the drip

Amino acid	Meat[1/] g/100 g protein	Drip[2/] g/100 g protein	Percentage of total contained in drip
Arginine	5.68	3.80	3.63
Histidine	3.13	4.24	7.26
Isoleucine	5.49	4.17	4.05
Leucine	8.83	6.87	4.15
Lysine	8.99	7.72	4.58
Methionine	2.41	1.84	4.07
Threonine	4.06	3.95	5.16
Valine	5.89	6.06	5.48
Average			4.79

1/ Microbiological assay.

2/ Amino acid analyzer.

Table 14. Essential amino acids in white chicken meat and drip after 90 days frozen storage and the percentage of the total contained in the drip

Amino acid	Meat 1/ g/100 g protein	Drip 2/ g/100 g protein	Percentage of total contained in drip
Arginine	4.31	4.36	9.63
Histidine	2.58	4.45	16.45
Isoleucine	4.76	4.12	8.25
Leucine	7.42	6.58	8.47
Lysine	7.91	7.94	9.61
Methionine	1.90	1.73	8.69
Threonine	3.12	3.86	11.83
Valine	4.91	5.75	11.18
Average			10.51

1/ Microbiological assay.

2/ Amino acid analyzer.

value for the eight amino acids was 4.79 percent.

The essential amino acids obtained in drip from white chicken meat after frozen storage of 90 days ranged from 8.25 percent for isoleucine to 16.45 percent for histidine (Table 14). Seven of the amino acids had concentrations in the range between 8.25 and 11.83 percent while the average amount of amino acid in the drip was 10.51 percent.

The amounts of specific amino acids (and ammonia), other than the essential amino acids, found in drip from dark and white chicken meat after frozen storage periods of 30 and 90 days are presented in Table 15. The results were expressed in terms of milligrams of amino acid per ml of drip collected (as reported in Table 3).

The quantities of all of the amino acids were larger in the drip from both the white and dark meat stored for 90 days than the quantities found in the samples from dark and white meat stored for 30 days. In addition, larger amounts of amino acids were detected in drip from white meat stored for both 30 and 90 days than from the drip from comparable dark meat samples.

The quantities of ammonia in the drip from dark and white meat after 90 days of frozen storage was slightly larger than that in the drip from dark and white meat frozen for 30 days. An increase in ammonia was also reported by Monzini (1953 b) in studies on the freezing and thawing of beef.

The averages of the total amino acids lost from both dark and white chicken meat held for frozen storage periods of 30 and 90 days are presented in Table 16. The results obtained from both microbiological assays and the amino acid analyzer are included.

Table 15. Quantity[1/] of specific amino acids and ammonia contained in the drip from dark and white chicken meat after frozen storage periods of 30 and 90 days

Amino acid	30 days storage		90 days storage	
	Dark (mg/ml)	White (mg/ml)	Dark (mg/ml)	White (mg/ml)
Alanine	2.2	3.0	3.8	4.9
Ammonia	0.5	0.3	0.7	0.6
Aspartic acid	3.1	4.5	6.0	7.4
Glutamic acid	4.3	6.4	7.6	7.9
Glycine	1.9	2.6	3.3	4.0
Proline	0.9	1.7	2.4	2.9
Serine	0.9	1.8	1.9	2.9

1/ Average of two samples determined with the amino acid analyzer and reported as milligrams of amino acid per milliliter of drip.

Table 16. Averages of the total amino acids lost[1/] from dark and white chicken meat after frozen storage periods of 30 and 90 days

Sample	Frozen storage (days)	Microbiological assay	Amino acid analyzer
		%	%
Dark	30	2.95	3.80
	90	7.58	8.01
White	30	4.11	4.79
	90	9.77	10.51

1/ Contained in drip.

A comparison of these results indicates that an increase in the percentage of amino acids in the drip was obtained from meat subjected to increased periods of frozen storage. This was evident in drip from both the dark and white chicken meat. Larger quantities of the essential amino acids were also obtained in the drip from frozen white meat than were obtained from frozen dark meat.

Results from both methods of assay were in general agreement as to the quantities of amino acids obtained from the dark and white meat samples and the increase in amounts of amino acids with increased periods of frozen storage (30 to 90 days). However, the results obtained with the analyzer were somewhat higher than those obtained by microbiological assays.

Some of the variation can be attributed to the different methods of analysis used. The loss of protein in the procedure for decolorizing the hydrolyzates should also be considered since a considerable loss of phenylalanine was indicated in this study. A similar loss of phenylalanine and an abundant loss of tyrosine was reported by Featherstone (1964). Some protein loss may have also occurred in preparing the drip hydrolyzates for analysis on the analyzer.

The amino acid values obtained by the microbiological assays were based on the protein contents of the original drip samples; the values obtained by the amino acid analyzer were based on the protein contents of the samples of drip hydrolyzates which were prepared for analysis on the analyzer. The loss in protein nitrogen is reflected in the differences in the values obtained by the methods of assay.

A number of workers have suggested that denaturation occurs in frozen stored meat as a result of freezing (Reay, 1933; Luijpen,

1957; Pearson et al. 1959; Khan et al. 1963). Some of the structural proteins which are normally insoluble in the drip fluids may be modified by denaturation so that their solubilities are affected. As a result, the possible loss of these complex substances in drip during thawing is increased. In this respect, Seagran (1958) and Howard et al. (1958) indicated a similarity between the low ionic extracts of muscle and drip by means of electrophoretic patterns and by ultracentrifuge studies.

Since the drip obtained in this study was hydrolyzed, the protein constituents of the drip are expressed in terms of their amino acid composition. An examination of Tables 6-9 indicates that, for many of the essential amino acids assayed, the percentages of the total amino acids contained in the drip were quite similar. Therefore, the relative proportions of the amino acids assayed in the drip are quite similar to the proportions of these amino acids present in the frozen meat. This may indicate, as Pearson et al. (1959) suggested, a breakdown or denaturation of the structural muscle proteins.

SUMMARY

The meat and drip from frozen heavy hens were studied to determine the quantities of amino acids present in the meat and the quantities of amino acids lost in the drip.

Optimum conditions of hydrolysis for amino acids in chicken meat were determined by studying the effects of time and method of hydrolysis on the liberation of arginine, leucine and methionine. The methods of hydrolysis compared were refluxing with 6N hydrochloric acid, autoclaving at 15 pounds pressure with 2N hydrochloric acid, and autoclaving in a sealed tube with 3N hydrochloric acid. The quantities of amino acids were determined by microbiological assay using L. mesenteroides as the test organism. Maximum liberation of the three amino acids was obtained in 16 hours.

Drip from chicken meat was hydrolyzed by refluxing with 6N hydrochloric acid; the optimum release of arginine, leucine and methionine was obtained between 8 and 16 hours of hydrolysis. A 16 hour hydrolysis period was used for both the chicken meat and the chicken drip.

Drip obtained from dark and white chicken meat after different periods of frozen storage was analyzed for protein content. The protein content of the drip obtained from white meat after 30 days (9.17 percent) and 90 days (10.92 percent) of frozen storage was higher than the content in the drip from dark meat after 30 days (5.16 percent) and 90 days (6.26 percent) of frozen storage. This indicates that the protein content of drip from frozen white meat is higher than the protein content of drip from frozen dark meat. These results also show that drip obtained from dark and white chicken meat after 90 days of

frozen storage contained more protein than the drip from meat held in frozen storage for 30 days.

The essential amino acids in acid and alkaline hydrolyzates of dark and white chicken meat and drip were determined by microbiological assay. L. mesenteroides was used for determining arginine, leucine, isoleucine, valine, lysine, histidine, methionine, and phenylalanine; L. arabinosus was used to determine tryptophane and threonine was determined using S. faecalis. Frozen acid hydrolyzates from drip were also analyzed on a Beckman/Spinco Amino Acid Analyzer.

The concentrations of the essential amino acids in the chicken meat decreased with extended frozen storage. This was characteristic of both the dark and white meat.

The concentrations of essential amino acids in the drip from dark and white chicken meat increased as the time of frozen meat storage was increased. In addition, larger quantities of the essential amino acids were detected in the drip from frozen white meat than from frozen dark meat after both periods of storage. These results were obtained by both the microbiological assay and the amino acid analyzer.

The quantitative results obtained from the analyzer were higher than those obtained by the microbiological assay. These differences in results are mainly attributed to the loss of protein nitrogen from the hydrolyzates on decolorizing for humin removal.

The relative proportions of amino acids assayed in the drip were similar to the proportions of these same amino acids in the frozen meat. These results indicate a possible denaturation of the structural muscle protein in the frozen stored chicken meat.

Under the conditions of this study, frozen chicken meat lost readily detectable quantities of protein nitrogen in the drip after thawing. The losses increased when the chicken meat was held for a longer period of time in frozen storage. This indicated that the practice of holding poultry for extended periods of frozen storage leads to protein breakdown and the subsequent loss of protein by leaching.

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APPENDIX A

Table 1. Standard amino acid solutions for microbiological assays

Amino acid [1/]	mg/1000 ml water	Concentration g/ml
L-Arginine	2.0	2000
L-Histidine	0.5	500
dL-Isoleucine	2.4	2400
L-Leucine	2.0	2000
L-Lysine [2/]	6.0	6000
L-Methionine	0.6	600
L-Phenylalanine	1.0	1000
L-Threonine	0.5	500
L-Tryptophane	0.4	400
L-Valine	1.0	1000

1/ Obtained from Nutritional Biochemical Corporation, Cleveland 28, Ohio.

2/ L-Lysine monohydrochloride was used at a level of 7.4982 g/100 ml water.

APPENDIX B

Sample calculation:

(μg/ml) X Dilution factor = μg/g of sample.

$\frac{\mu g/g}{\%\ \text{protein}}$ X 100 = μg/g protein.

(μg/gm) X 10^{-4} = g/100 g protein.

APPENDIX C

Table 1. Quantities of arginine liberated from dark chicken meat after hydrolysis by different methods for specific periods of time as determined by microbiological assay

Method	Time (hrs)	µg/ml	g/100 g protein
Refluxing	1	13.78	3.89
	2	16.88	4.77
	4	17.70	5.00
	8	18.32	5.18
	16	18.86	5.33
	24	18.03	5.09
Autoclaving in flask	1	12.92	3.65
	2	13.94	3.76
	4	13.28	3.94
	8	14.89	4.21
	16	16.25	4.59
	24	15.05	4.25
Autoclaving in sealed tube	1	10.90	3.08
	2	11.86	3.35
	4	14.40	4.07
	8	15.36	4.33
	16	15.37	4.33
	24	15.61	4.41

Table 2. Quantities of leucine liberated from dark chicken meat after hydrolysis by different methods for specific periods of time as determined by microbiological assay

Method	Time (hrs)	µg/ml	g/100 g protein
Refluxing	1	19.55	5.52
	2	19.83	5.60
	4	22.45	6.34
	8	24.18	6.83
	16	25.24	7.13
	24	23.46	6.63
Autoclaving in flask	1	13.79	3.90
	2	16.86	4.76
	4	19.17	5.42
	8	20.86	5.89
	16	22.84	6.45
	24	22.64	6.40
Autoclaving in sealed tube	1	13.55	3.83
	2	14.97	4.23
	4	19.33	5.46
	8	21.19	5.98
	16	22.59	6.38
	24	21.92	6.19

Table 3. Quantities of methionine liberated from dark chicken meat after hydrolysis by different methods for specific periods of time as determined by microbiological assay

Method	Time (hrs)	µg/ml	g/100 g protein
Refluxing	1	6.23	1.76
	2	6.83	1.93
	4	7.53	2.13
	8	8.17	2.31
	16	7.98	2.25
	24	6.81	1.92
Autoclaving in flask	1	4.51	1.27
	2	5.38	1.52
	4	6.55	1.85
	8	7.07	1.99
	16	6.62	1.87
	24	6.41	1.81
Autoclaving in sealed tube	1	4.12	1.16
	2	4.21	1.19
	4	5.74	1.62
	8	6.96	1.97
	16	6.48	1.83
	24	6.11	1.73

APPENDIX D

Table 1. Quantities of specific amino acids liberated from dark chicken drip after hydrolysis (refluxing with 6N HCl) for specified periods of time, as determined by microbiological assay

Amino acid	Time (hrs)	µg/ml	g/100 g protein
Arginine	2	34.38	2.63
	4	39.75	3.04
	8	47.00	3.59
	16	47.37	3.62
Leucine	2	10.80	4.26
	4	11.22	4.42
	8	13.38	5.28
	16	14.13	5.57
Methionine	2	8.15	1.29
	4	8.65	1.36
	8	10.41	1.65
	16	11.41	1.80

APPENDIX E

Table 1. Essential amino acid concentrations[1/] contained in chicken meat after frozen storage periods of 30 and 90 days

Amino acid[2/]	Storage (days)	Dark meat		White meat	
		A	B	A	B
Arginine	30	6.04	6.11	5.58	5.78
	90	5.11	5.53	4.14	4.48
Histidine	30	2.30	2.18	3.12	3.13
	90	1.45	1.86	2.51	2.64
Isoleucine	30	5.19	5.40	5.44	5.54
	90	4.29	4.71	4.69	4.84
Leucine	30	8.45	8.70	8.77	8.88
	90	6.57	7.55	7.19	7.65
Lysine	30	8.84	8.73	8.99	8.99
	90	8.08	7.86	7.76	8.05
Methionine	30	2.52	2.42	2.47	2.35
	90	1.77	2.09	1.77	2.03
Phenylalanine	30	0.81	0.81	0.47	0.67
	90	0.31	0.35	0.20	0.25
Threonine	30	4.17	4.09	4.06	4.07
	90	2.05	3.16	2.95	3.30
Tryptophane	30	1.16	1.20	1.30	1.37
	90	1.10	1.04	1.25	1.30
Valine	30	5.13	4.95	5.98	5.81
	90	4.59	4.83	4.83	5.00

1/ Average concentration in g/100 g protein of two replicates.

2/ Microbiological assay.

Table 2. Essential amino acid concentrations[1/] contained in drip from dark and white chicken meat after frozen storage periods of 30 and 90 days as determined by microbiological assay

Amino acid	Storage (days)	Dark meat		White meat	
		A	B	A	B
Arginine	30	2.22	3.19	3.30	3.30
	90	2.70	3.18	3.87	3.12
Histidine	30	1.48	1.24	3.13	3.33
	90	1.98	2.00	3.79	3.29
Isoleucine	30	2.59	2.93	4.22	4.30
	90	3.42	3.70	5.40	4.83
Leucine	30	5.56	5.62	6.37	6.17
	90	5.90	6.21	6.84	5.88
Lysine	30	4.98	5.23	6.22	6.27
	90	5.82	6.46	7.21	6.98
Methionine	30	1.09	1.37	1.50	1.48
	90	1.44	1.38	1.80	1.66
Phenylalanine	30	0.13	0.14	0.18	0.21
	90	0.15	0.15	0.22	0.25
Threonine	30	2.33	2.36	3.17	3.19
	90	2.77	3.04	3.22	3.21
Tryptophane	30	0.94	0.97	0.99	1.05
	90	1.22	1.64	1.25	1.28
Valine	30	4.64	5.30	5.36	5.76
	90	5.21	6.06	6.23	6.71

1/ Average concentration in g/100 g protein of two replicates.

Table 3. Essential amino acid concentrations[1/] contained in drip from dark and white chicken meat after frozen storage periods of 30 and 90 days as determined by amino acid analyzer

Amino acid	Storage (days)	Dark meat		White meat	
		A	B	A	B
Arginine	30	2.82	4.61	4.14	3.45
	90	2.90	3.43	4.89	3.82
Histidine	30	2.34	3.54	4.18	4.30
	90	2.55	2.86	4.92	3.98
Isoleucine	30	2.92	3.19	3.93	4.41
	90	3.06	3.56	4.42	3.82
Leucine	30	6.30	6.36	6.48	7.26
	90	6.16	6.86	6.72	6.44
Lysine	30	7.38	7.62	7.32	8.11
	90	7.60	8.13	7.73	8.15
Methionine	30	1.68	1.45	1.75	1.92
	90	1.25	1.86	1.86	1.59
Threonine	30	2.98	2.97	3.54	4.36
	90	2.89	3.55	3.70	4.01
Valine	30	5.09	5.31	5.72	6.40
	90	5.41	5.86	5.74	5.75

1/ Concentration in g/100 g protein.

APPENDIX F

Table 1. Concentrations[1/] of specific amino acids contained in drip from dark and white chicken meat after frozen storage periods of 30 and 90 days as determined by amino acid analyzer

Amino acid	Storage (days)	Dark meat		White meat	
		A	B	A	B
Alanine	30	5.72	5.88	6.01	6.73
	90	5.89	5.93	6.12	6.15
Ammonia	30	1.29	1.17	0.63	0.66
	90	1.26	1.10	0.84	0.82
Aspartic acid	30	8.18	8.13	8.61	10.14
	90	8.30	9.80	8.88	9.25
Glutamic acid	30	11.81	10.81	9.65	10.89
	90	10.64	12.20	9.64	9.62
Glycine	30	4.84	4.91	5.22	5.57
	90	4.81	5.31	4.93	5.01
Proline	30	3.73	3.68	3.45	3.84
	90	3.74	3.65	3.58	3.68
Serine	30	2.38	2.29	3.30	4.08
	90	2.25	3.43	3.24	3.78

1/ Concentration in g/100 g protein.